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THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS.*

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Plates 44 to 47.

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The demonstration of the complete dependence of the spontaneous liberation of epinephrin from the adrenals upon the integrity of their nerve supply naturally raises the question where the central mechanism which sustains this secretion is situated. We are not aware of the existence of evidence upon this point. Elliott's statement[1] that exhaustion of the store of epinephrin in the adrenals by electrical excitation of afferent nerves does not occur if the cord is transected anywhere below the level of the vasomotor center in the medulla oblongata, but does occur when transection of the brain-stem is made just above the anterior corpora quadrigemina, has no direct bearing on the question. For it has not been proved that the exhaustion of the store is due entirely or mainly to increase in the rate of liberation of epinephrin, on which Elliott made no observations. A change in the amount of the store of epinephrin would merely show that some alteration had occurred in the relation between the rate of formation and the rate of discharge of the epinephrin. Nor would such observations even if they were accepted as proving an increase in the liberation brought about reflexly through a center in the bulb or higher up, give any indication whether the steady spontaneous liberation of epinephrin is sustained from a center at this level. Absence of effect

* A note on this work was published in the *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 143.

[1] Elliott, T. R., *J. Physiol.*, 1912, xliv, 407.

of afferent stimulation upon the epinephrin store after section of the cord just below the bulb likewise affords no indication whatever that the rate of spontaneous liberation of epinephrin has been interfered with. As a matter of fact, we have found in cats that after transection of the cord at various levels in the cervical region, the secretion of epinephrin into the blood, far from being abolished, proceeds without interruption. The rate of liberation may even remain sensibly the same, within the limits of error of the methods used for estimating it, as before the section. This is illustrated in Experiment 1, in which adrenal blood was obtained from a cava pocket before and after section of the cord opposite the body of the fourth cervical vertebra, between the fourth and fifth cervical segments.

Experiment 1. Condensed Protocol.—Cat; weight 2.31 kilos.

The animal received 4 gm. of urethane by stomach tube at 12.30 p.m., and 2 gm. at 2.00 p.m.

2.15 p.m. Tracheal and jugular cannulas were inserted and a specimen of jugular blood was obtained. The cord was exposed for about a segment in the midcervical region. A short cava pocket was then made, all the arteries (renal, celiac, and superior mesenteric, and abdominal aorta) being tied. Artificial respiration was started while the animal was breathing well spontaneously, and collection of blood from the adrenals begun.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	gm.		*gm.*
1	2	20 sec.	6.6
2	10	2 min.	5.0
3	2.8	2 " 30 "	1.2
4	2.9	4 "	0.7
5	2.4	4 " 15 "	0.6
6	4.5	6 " 10 "	0.7
7	3.3	7 " 10 "	0.5
8	2.1	7 " 10 "	0.3

After collection of the second adrenal specimen the cord was cut completely at the level of the body of the fourth cervical vertebra, between the fourth and fifth segments, as verified post mortem. After collection of the sixth adrenal specimen it was verified that the section of the cord was complete. After collection of the eighth specimen, while the pocket was still clipped off, blood was obtained from the abdominal aorta. The proportion of corpuscles in the blood (hematocrit) was 25.5 per cent. Combined weight of adrenals 0.440 gm.

In Figs. 1 to 3 are reproduced some of the tracings from the rabbit intestine and uterus segments on which the blood specimens were tested. At 2 (Fig. 1) the Ringer's solution in which the intestine segment was beating was replaced by indifferent (jugular) blood, and this at 3 by the second adrenal blood specimen, collected just before transection of the cord. The inhibition of the intestine, and therefore the concentration of epinephrin in the blood, was obviously much less than with the sixth adrenal specimen, collected after section of the cord (Observation 7). The inhibition produced by the sixth specimen was less than that produced by the eighth (Observation 10). The progressive increase in the concentration is associated with the progressive slackening in the blood flow in successive samples. This is a phenomenon always observed in animals with intact central nervous system when the rate of flow happens to be diminishing, and it is undoubtedly due to the fact that the rate of liberation of epinephrin per unit of time remains approximately constant, at least for considerable periods, under the experimental conditions. The mere inspection of these tracings is of itself sufficient to show that section of the cord at the level mentioned cannot have caused any very great change in the rate of liberation of epinephrin. Figs. 2 and 3 are some of the tracings taken to assay the concentration of epinephrin in the second adrenal blood specimen and in the eighth specimen, collected respectively before and after the cord section. The adrenalin used for the epinephrin assays was always freshly assayed colorimetrically. It was found that the concentration in the second specimen was approximately 1:13,000,000 (Fig.2, Observations 36 and 38), an unusual degree of dilution corresponding to the unusually high rate of blood flow, associated with the high blood pressure before division of the cord, which is generally seen when all the arteries mentioned in the protocol have been ligated. The concentration in the eighth specimen was much greater than 1: 1,600,000, and slightly less than 1: 800,000 (Fig. 3, Observations 20, 22, and 24). The blood flow after section of the cord dropped abruptly, owing to paralysis of vasoconstrictors in spite of the previous ligation of arteries. It will be noted that the blood flow during collection of the eighth specimen was only one-sixteenth or one-seventeenth as great as during collection of the second specimen, while the concentration of epinephrin in the eighth specimen

was approximately sixteen times as great as in the second. In other words, the output of epinephrin (0.0004 mg. per minute) was not altered by the cord section.

It might be objected that the small blood flow during collection of the eighth specimen was not sufficient to prevent partial asphyxia of the adrenals, and that by some direct effect of this condition on the cells of the medulla an abnormal liberation of epinephrin, not mediated through the nervous system, took place. This objection is entirely without weight. For with even smaller flows through adrenals whose innervation has been interrupted no such liberation occurs.[2] After all, a flow of about 100 gm. of blood per 100 gm. of tissue, although small for the adrenal, is scarcely a starvation allowance.

It might much more plausibly be argued that the calculated output of epinephrin for the eighth specimen is likely to be less than the true rate after section of the cord, provided that the blood flow had not diminished. For it is quite unusual to find under any circumstances in adrenal blood collected in a cava pocket a much greater concentration than 1:1,000,000. If, then, with a declining rate of blood flow the maximum possible concentration has once been reached, the rate of liberation calculated for smaller flows will be less than the gland is capable of sustaining under the given experimental conditions with a more copious flow of blood. However, it is only when the calculated rate of liberation for the smaller flow is much less than for the larger flow, that any such question could arise. When with a relatively small blood flow the calculated output of epinephrin per minute is as great as with a larger flow all our experience goes to show that the rate of output calculated from the concentration and the blood flow can be legitimately compared for a wide range of blood flows lying above this smallest flow.

In the next experiment, the cord was transected at a slightly higher level between the third and fourth cervical vertebræ, through the fourth segment just above the origins of the fourth pair of cervical nerves, as shown at autopsy. The spontaneous liberation of epinephrin was studied by means of the eye reactions, the superior

[2] Stewart, G. N., and Rogoff, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 145; Marine, D., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916–17, ix, 1.

cervical ganglion having been previously excised. The eye reactions were employed because they afforded a convenient means of investigating the effects of section of the nerves going to the adrenals upon the spontaneous liberation after section of the cervical cord.

Experiment 2. Condensed Protocol.—Cat; weight 2.19 kilos. Left superior cervical ganglion excised 6 days before the experiment.

9.30 a.m. 4 gm. of urethane given by stomach tube.

10.30 a.m. Tracheal cannula inserted and cervical cord exposed between the third and fourth vertebræ. Cava pocket made, abdominal aorta and renal vessels being tied.

11.15 a.m. Started artificial respiration.

Time.		Duration of pocket.	Pupil dilatation.
a.m.			
11.20	Pocket experiment.	2 min., 40 sec.	None.
11.25	" "	5 "	Small; in 40–45 sec.
11.32	" "	4 " 30 "	" " 31 sec.
11.45	Spinal cord cut just above body of fourth cervical vertebra.		
11.46	Pocket experiment.	6 min., 40 sec.	Fair; in 34 sec.
11.55	" "	4 " 15 "	Small; " 36 "
p.m.			
12.02	" "	4 " 15 "	" " 35 "
12.10	Tied intestinal arteries; cut major and minor splanchnics on both sides in abdomen.		
12.15	Pocket experiment.	6 min., 15 sec.	Small; in 38–40 sec.
12.25	" "	6 " 12 "	" " 43 sec.
12.35	Cut both sympathetics in thorax (including splanchnics) below last rib.		
12.40	Pocket experiment.	6 min., 10 sec.	None.
12.50	" "	10 " 30 "	"

The pocket filled very slowly throughout the experiment; the heart was feeble.

In this experiment, as will be seen from the protocol, section of the cord at the level mentioned caused no noteworthy change in the eye reaction, certainly no clear diminution. The circulation was poor both before and after the section. The cava pocket filled slowly and the interval between the release of the pocket and the beginning of

the pupil dilatation was correspondingly long. Yet approximately the same time of collection was required to evoke a reaction of given magnitude before and after section of the cord. After division of the splanchnics in the abdomen and the sympathetic trunks in the thorax no eye reactions could be obtained, even with much longer periods of collection in the pocket; that is, the liberation of epinephrin after section of the cord must have been sustained from some part of the cord below the fourth cervical segment, and through the same nerves as with intact central nervous system.

It may be explained here that even when the cava pocket has been kept closed for so long a period that it is unable to receive any more adrenal vein blood, there is good evidence that the output of epinephrin still goes on into the blood of the adrenal capillaries at an approximately constant rate; and that when the pocket is released the full effect of this epinephrin is exerted in eliciting the eye reactions or increasing the blood pressure, just as if the blood had actually passed into the pocket. So that in the course of an experiment, the reactions evoked when a pocket clipped off for a given length of time is released are substantially of the same magnitude, whether the pocket has been overfilled or underfilled in that time. Of course, the interval after which the reactions occur is greatly influenced by the rate of the circulation, since the epinephrin-containing blood released from the pocket will take longer to reach the reacting structures with a slow than with a rapid blood flow. With a very slow flow, also, the blood collected in a pocket may be so small in amount that it is not promptly or completely passed into the circulation on release of the pocket. It is further to be expected that below a certain rate of flow the function of the adrenal medullary cells, as already suggested, will be interfered with. In that case, the steadiness, for given conditions, in the output of epinephrin per unit of time, which is so strikingly manifested over a wide range in the rate of the blood flow, will no longer be maintained. Our experience shows, however, that this point is not easily reached.

In the next experiment transection was made about two segments lower in the cervical cord, in order to localize more sharply the level of the cord concerned in the spontaneous secretion of epinephrin.

Experiment 3. Condensed Protocol.—Cat; weight 3.175 kilos. Recent parturition. Left superior cervical ganglion excised 1 week before the experiment.

9.45 a.m. 4 gm. of urethane.

10.45 a.m. Tracheal cannula inserted and cervical cord exposed between fifth and sixth vertebræ. Cava pocket prepared; intestinal and renal arteries and abdominal aorta tied.

11.30 a.m. Left pupil wider than right, both nictitating membranes forward; cat breathing quite well, but for uniformity of observations artificial respiration was started.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
a.m.				
11.40	Pocket experiment.	1 min., 30 sec.	Doubtful.	None.
11.42	" "	2 " 30 "	None.	"
11.45	" "	3 " 45 "	"	"
11.50	" "	5 "	"	"
p.m.				
12.05	Pocket experiment.* Cord stimulated with needle electrodes one segment below the exposed part for 3 min.	3 "	"	"
12.15	Left sympathetic and vagus cut in neck.			
12.17	Right sympathetic cut in neck.			
12.20	Pocket experiment.	5 min., 30 sec.	Very good; in 10 sec.	Very good; 10 sec.
12.26	" "	1 "	Good; in 15 sec.	Good; 15 sec.
12.30	" "	2 "	Very good; in 10.8 sec.	Very good; 10.8 sec.
12.37	Spinal cord cut just below body of fifth cervical vertebra.			
12.41	Pocket experiment.	2 min.	Very good; in 15 sec.	Very good; 15 sec.
12.45	" "	1 "	Good; in 18.6 sec.	Good.

* With each period of stimulation both pupils dilated instantaneously, and proportionally to the same extent, but the left pupil still remained wider than the right. After section of both cervical sympathetics, stimulation of the cord still caused dilatation of both pupils. At autopsy it was found that the cord had been cut between the fifth and sixth cervical segments.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
p.m.				
12.50	Major and minor splanchnics in abdomen cut on both sides.			
12.53	Pocket experiment.	2 min.	None.	None.
12.56	" "	5 "	Slight; in 20–25 sec.	"
1.05	Right semilunar ganglion excised; nerves coming to left ganglion cut. Circulation getting feeble.			
1.12	Pocket experiment.	5 min., 30 sec.	None.	None.
1.25	" "	9 " 30 "	Slight; in 60–90 sec.	Slight.
1.47	" "	24 "	Slight; in 40–45 sec.	
2.25	" "	25 "	None.	None.
3.05	" " with massage of adrenals.	15 "	"	"
3.30	Injected 0.5 cc. of 1: 1,-600,000 adrenalin.		"	"
3.35	Injected 0.5 cc. of 1: 270,000 adrenalin.		Slight; in 40–50 sec.	Slight.

In this animal the denervated eye reactions were employed and an interesting preliminary observation was made upon them without some reference to which the first part of the protocol would probably appear as puzzling to the reader as the observations did to us when they were being made. The left superior cervical ganglion had been excised a week before the experiment. The eye reactions ought, therefore, to have been easily obtained in the pocket experiments made between 11.40 and 12.05 before section of the cord. In the numerous observations made by us, we have, apart from this experiment, scarcely ever had a negative result, especially with a duration of occlusion of the pocket as long as 5 minutes, and with the good blood flow and satisfactory filling of the pocket which existed in this cat. It was conceivable, of course, that for some reason the adrenals might have been giving off much less epinephrin than usual; or that the reactions of the iris, etc., might have been unduly depressed by

the anesthetic, although there was nothing in the behavior of the animal to indicate that the urethane, a very uniform anesthetic, as is well known, had affected this animal at all differently from any of the others. Desiring to increase the output of epinephrin to the maximum, we stimulated the cord with needle electrodes, hoping thus to strike the secretory path, but again with a negative result as regards the eye reaction. Even with the collection of adrenal blood in the pocket for as long as 3 minutes during stimulation no eye reactions were obtained. Stimulation of the cord, however, caused immediate dilatation of the pupils of both eyes. This dilatation was still elicited after section of both vago-sympathetics in the neck, and may be attributed to stimulation of afferent fibers in the cord. But the interesting point was that after section of the cervical sympathetics excellent eye reactions were now evoked with collections of adrenal blood in the cava pocket much shorter than those which gave a negative result before division of the nerves. The most probable conclusion would seem to be that some small part of the superior cervical ganglion had escaped excision, and that the innervation of the eye through the cervical sympathetic was not entirely interrupted, although it was impossible to verify this at autopsy on account of scar tissue. The change in the sensitive structures of the iris and nictitating membrane, on which the increased power of reaction to adrenalin depends, must be assumed to have developed as usual after removal of the ganglion, although prevented from manifesting itself, even in the presence of a quantity of epinephrin more than sufficient to evoke good reactions, until the control of the remaining sympathetic fibers was removed.

It will be seen from the protocol that after section of the cord between the fifth and sixth cervical segments excellent pupil and nictitating membrane reactions were still obtained. The reactions were not noticeably less for equal periods of collection of blood in the pocket than before the section, although the interval after which they occurred was somewhat lengthened, corresponding to the slower blood flow. Subsequent division of the major and minor splanchnics in the abdomen greatly weakened the reactions and increased the time of collection necessary to elicit even a feeble response. Further section of fibers coming to the adrenals abolished the reactions even

with very long periods of collection. This was not due to total loss of sensitiveness of the reactive structures in the eye. For even at the end of the experiment a slight but definite response was still obtained on injection of 0.002 mg. of adrenalin.

As a first approximation towards defining the lower limit of the region of the cord concerned in the spontaneous liberation of epinephrin the following experiment was performed, both eye reactions and rabbit segment tests being employed.

Experiment 4. Condensed Protocol.—Cat; weight 1.72 kilos. Left superior cervical ganglion excised 8 days before the experiment.

10.00 a.m. 3.5 gm. of urethane.

11.20 a.m. Tracheal cannula inserted. Cervical and dorsal cord exposed for about a segment at each point. Long cava pocket made and arteries (renal, celiac, mesenteric, and abdominal aorta) tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
p.m.				
12.15	Pocket experiment.	1 min., 10 sec.	Good; in 8.8 sec.	Positive; 11 sec.
12.18	" "	2 "	Very good; in 8.8 sec.	Very good; 8.8 sec.
12.22	Cut cord between fifth and sixth cervical vertebræ.			
12.32	Pocket experiment.	1 min., 45 sec.	Very good; in 10.8 sec.	Very good (a little later).
12.35	" "	2 "	Very good; in 11.2 sec.	Very good (a little later).
12.40	Cut cord at fourth dorsal vertebra.			
12.49	Pocket experiment.	2 min.	None.	None.
12.55	" " In the last two pocket observations the filling was slower than before.	3 "	"	"
1.07	Pocket experiment with stimulation of cord between fifth and sixth dorsal vertebræ.	3 min.	Very good; in 20.2 sec.	Very good; 20.2 sec.

1.20 p.m. Put cannula in lower end of cava, making a short pocket, and collected two specimens of adrenal blood. First specimen, 1.6 gm. in 5 min.,

45 sec. (0.3 gm. per min.); second specimen, 3.7 gm. in 20 min. (0.2 gm. per min.). With the pocket still clipped off, blood was obtained from the abdominal aorta.

The left adrenal weighed 0.214 gm. and contained 0.12 mg. of epinephrin; the right adrenal weighed 0.238 gm. and contained 0.12 mg. of epinephrin.

The experiment shows that after transection of the cord between the fifth and sixth cervical vertebræ (through the fifth cervical segment, just below the fifth pair of nerve roots, as found at autopsy) the eye reactions were elicited by the adrenal blood, apparently in the same strength for a given time of closure of the cava pocket as before the section, although the interval between release of the pocket and the beginning of the pupil dilatation was somewhat lengthened, as would necessarily be the case owing to the lowered blood pressure and diminished speed of the blood. When the cord was now cut between the fourth and fifth dorsal vertebræ (between the third and fourth segments, as shown at autopsy) no eye reactions could be obtained even with longer periods of occlusion of the pocket than sufficed to cause excellent reactions just before. That the negative result was not due to diminished blood flow, but that the adrenals were still capable of secreting epinephrin actively, was proved by stimulating the cord electrically by needle electrodes inserted one above the fifth, and the other above the sixth dorsal spine while the adrenal blood was being collected in the pocket. Very good eye reactions followed the opening of the pocket, naturally after a longer interval than before the dorsal section, corresponding to the slower blood flow. The adrenal blood specimens now drawn off were so small that it was not quite certain whether some of the epinephrin in them might not have been liberated during the manipulations in inserting the cannula. Despite this, however, the concentration found even in the first specimen was somewhat less than 1: 17,000,000, corresponding to an output of epinephrin per minute of 0.00002 mg.; that is, far below any concentration or output ever met with in the cat with intact adrenal innervation.

The experiments next to be considered, in which the animals were allowed to survive 2 or 3 days after the cord section[3] before the epinephrin output was tested, so that any possible irritative discharge

[3] All the operations were performed under ether anesthesia.

might be eliminated, yield clear evidence that transection of the dorsal cord at the level mentioned in the last experiment, or even a segment higher reduces the rate of liberation almost to zero. Transection one segment lower abolished the liberation entirely, or at least reduced it so much that no epinephrin could be detected in the adrenal blood by sensitive rabbit intestine and uterus segments. The upper limit of the portion of the cord related to the spontaneous liberation of epinephrin was also further defined by survival experiments and these may be taken first.

Experiment 5. Condensed Protocol.—Cat; weight 2.17 kilos. Cord transected at the level of the body of the last cervical vertebra 3 days before the experiment. Animal in fairly good condition. Anesthetized with ether for the insertion of the tracheal cannula; thereafter no more ether was required as the operative field was, of course, absolutely anesthetic because of the previous spinal cord section. Cava pocket made with ligation of all the usual arteries. Cannula inserted in lower end of pocket (short pocket) and the following samples of adrenal blood collected.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	gm.		*gm.*
1	2.4	4 min., 50 sec.	0.5
2	2.2	7 " 30 "	0.3
3	2.1	9 "	0.23
4	2.2	17 " 30 "	0.13

While the pocket was still clipped off blood was obtained from the abdominal aorta. While the pocket was being tied off some blood was left in it and the first adrenal specimen was therefore somewhat diluted. The autopsy showed that the cord had been divided between the last cervical and the first thoracic segments. Combined weight of adrenals 0.362 gm.

In survival experiments it is not so easy as in acute experiments to decide whether transections of the cervical cord leave the rate of liberation of epinephrin unaltered, or somewhat diminish it because the rate before and after the section cannot be compared on the same animal within a short interval of time. All that can be done is to determine whether the residual output after the cervical section is within the range established for animals under the same experimental conditions, but with intact central nervous system. In Experiment

5, as will be seen from the specimens of rabbit intestine and uterus tracings reproduced in Figs. 4 to 6, concentrations of epinephrin within the normal range[2] were found in the adrenal blood samples collected after section of the cervical cord between the last cervical and the first thoracic segments.

The adrenalin assays on the intestine segments gave a concentration, in the fourth adrenal specimen, of about 1: 1,500,000 (Fig. 4, Observation 19; Fig. 5, Observation 27); and in the second adrenal specimen a concentration greater than 1: 2,500,000 and less than 1: 1,500,000 (Fig. 4, Observation 21; Fig. 5, Observations 29 and 27). Taking the concentration in the second specimen as 1: 2,000,000, we get a liberation of epinephrin per minute of not quite 0.0002 mg. (0.0001 mg. per kilo of body weight). It may be considered certain that the output which the glands would have been capable of maintaining with a more nearly normal blood flow was at any rate not less than this. It might have been more, since as already mentioned, we rarely encounter concentrations of more than 1: 1,000,000 in adrenal blood collected under our experimental conditions; and therefore the point might already have been passed at which the declining blood flow can be compensated by increased concentration of epinephrin.

The fact that it was not necessary to administer an anesthetic in order to collect the adrenal blood in this animal, since the cord section had rendered the operative field totally insensitive, has probably no bearing on the question whether the rate of output was somewhat diminished by the cervical section. For although some writers have assumed that anesthetics markedly increase the rate of liberation of epinephrin, there is no real proof of this. In any case, if the anesthetic exerts its effect through the higher parts of the central nervous system, the administration of an anesthetic could not have increased the output of epinephrin in this animal. In reference to the experiments (Nos. 1 to 4) in which the epinephrin output was determined immediately after section of the cervical cord, it might be asked, however, whether the anesthetic had not already abolished the activity of any portion of the brain or bulb which might be related to the epinephrin secretion. Division of the cervical cord would in that case cause no diminution in the output of epinephrin if made above the level of the spinal center. An assumption equally plausible, but at present equally devoid of experimental basis, is that anesthetics abolish or lessen an inhibition of the spinal center from a center in the brain. On this hypothesis, the output of epinephrin seen after cervical cord section would be considered as greater, not smaller, than the normal output with intact central nervous system.

The progressive increase in the concentration of epinephrin in successive adrenal blood samples associated with gradual diminution in the rate of blood flow is well brought out in the intestine tracings reproduced in Fig. 4, and still better in the uterus tracings in Fig. 6. This phenomenon and what underlies it—the stability in the rate of epinephrin discharge—is so characteristic when adrenal blood is collected with intact central nervous system, that its occurrence after spinal section lends support to the conclusion that the secretion of epinephrin when the connection of the cord with the brain has not been interrupted is also sustained largely, if not entirely, from the cord.

In the next experiment the eye reactions were studied in a cat 2 days after section of the cord just above the body of the seventh cervical vertebra.

Experiment 6. Condensed Protocol.—Cat; weight 2.825 kilos. Left superior cervical ganglion excised 1 week, and spinal cord divided 2 days before the experiment, just above the body of the last cervical vertebra.

10.00 a.m. Anesthetized with ether while the tracheal cannula was being inserted; thereafter no more ether was required as the operative field was necessarily absolutely insensitive because of the previous section of the cord.

10.05 a.m. The abdomen was opened and the cava pocket made, all the usual arteries being tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
a.m.				
10.28	Pocket experiment.	1 min.	Small; in 20 sec.	Small (shortly after pupil).
10.30	" "	2 "	Good; in 15 sec.	Good; 15 sec.
10.45	Injected 0.5 cc. (1: 330,000) of adrenalin into jugular vein.		Very good; in 15 sec.	Very good; 15 sec.
10.50	Injected 0.25 cc. (1: 330,000) of adrenalin into jugular vein. The eye reactions after this injection were about the same or slightly less than in the observation at 10.30 a.m.		Good; in 17.2 sec.	Good; 17.2 sec.
10.55	Pocket experiment.	1 min.	Very slight; in 25 sec.	None.
10.58	" " The reactions were about the same as at 10.50 a.m.	2 "	Good; in 21 sec.	Good; 21 sec.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
a.m.				
11.02	Pocket experiment. The reactions were about the same as at 10.45 a.m.	3 min., 40 sec.	Very good; in 16.8 sec.	Very good; 16.8 sec.
11.10	Cut nerves to both semilunar ganglia in abdomen.			
11.20	Pocket experiment. Pocket not so well filled as before.	3 min.	None.	None.
11.25	Pocket experiment. The reaction was about the same as at 10.58 a.m.	5 "	Distinct; in 23.4 sec.	Distinct.
11.30	Both semilunar ganglia extirpated.			
11.37	Pocket experiment.	5 min.	None.	None.
11.45	" " Pocket filled very slowly.	8 "	Slight; in 41 sec.	"
11.55	Cut lumbar sympathetic chain just below diaphragm.			
12.00	Pocket experiment. Very poor flow.	25 min.	Slight; in 90 sec.	None.
p.m.				
12.50	Pocket experiment. Better flow.	10 "	Slight; in 60 sec.	"

The autopsy showed that the cord was divided through the seventh cervical segment, immediately below the origins of the seventh pair of nerves.

The fact that adrenal blood collected in the cava pocket caused good eye reactions was ascertained. It was shown by the injection of adrenalin solution that the amount of epinephrin secreted per minute was about 0.0004 mg. (0.00015 mg. per kilo of body weight per minute); this is a substantial output, although considerably less than the average, as estimated by eye reactions, in cats with intact central nervous system.[4] Various nerves going to the adrenals were then divided and the effect in diminishing the eye reactions was noted. After division of the fibers coming to the semilunar ganglia, the eye reactions elicited by adrenal vein blood collected in the cava pocket were markedly diminished. A 3 minute collection gave no reactions, whereas before the nerve section a 1 minute collection caused a slight

[4] Stewart and Rogoff, *J. Pharm. and Exp. Therap.*, 1916–17, ix, 479.

effect on the pupil, and a 2 minute collection good dilatation of the pupil and retraction of the nictitating membrane. After removal of both semilunar ganglia, which, of course, insured the section of any strands coming to the ganglia overlooked in the previous section, a 5 minute collection of adrenal blood caused no eye reactions, although previously a 2 minute collection gave good reactions. On release of a pocket occluded for 8 minutes, a small dilatation of the pupil was obtained.

In connection with the fact that slight eye reactions were still elicited with long periods of closure of the cava pocket even after extensive section of possible nerve paths to the adrenal glands, it must be pointed out that these nerve sections entail considerable manipulation of, and in the neighborhood of, the adrenals. With the slow blood flow toward the end of the experiment, epinephrin liberated by massage would take long to be completely washed out. It may be concluded that even the feeble reactions obtained after these nerve sections were not due entirely to genuinely secreted epinephrin. Survival experiments published elsewhere[2] have shown that the epinephrin output after section of the adrenal nerves is either abolished or reduced so much as to be incapable of detection by sensitive rabbit intestine and uterus segments. On the other hand, in acute experiments after the same nerve sections, although the output of epinephrin is greatly reduced, a content capable of detection is usually still found in adrenal blood.

It may, therefore, be confidently assumed that the whole output of epinephrin from the adrenals after section of the cervical cord near its lower limit is mediated through the same nerves which are concerned in the liberation with intact nervous system.

The last two survival experiments to be quoted were made for the purpose of defining more exactly the lower limit of the spinal region concerned in the epinephrin secretion.

Experiment 7. Condensed Protocol.—Cat; weight 1.59 kilos. Left superior cervical ganglion excised 6 days before the experiment and spinal cord transected between the fifth and sixth thoracic vertebræ 3 days before the experiment. Condition good. The autopsy showed cord section between the fifth and sixth thoracic segments.

2.00 p.m. Ether was given while the tracheal and jugular cannulas were

inserted. Thereafter no more ether was required as the operative field was, of course, totally insensitive owing to the cord section. A specimen of jugular blood was obtained. A long cava pocket was prepared; the renal arteries and abdominal aorta were tied.

2.45 p.m. Pocket experiment, 1 minute, 25 seconds. No eye reactions.

2.48 p.m. Pocket experiment, 3 minutes, 20 seconds. No eye reactions.

The blood flow was good; the pocket filled well.

3.00 p.m. Intestinal arteries tied. Cannula put in lower end of pocket (now made into a short pocket). Adrenal blood specimens collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	gm.		*gm.*
1	1.5	1 min., 25 sec.	1.0
2	3.2	4 " 30 "	0.7
3	3.5	10 "	0.35
4	2.2	10 "	0.22

Through a cannula in the jugular vein inserted under ether anesthesia the following injections were made: 0.5 cc. (1: 500,000) of adrenalin; good pupil and nictitating reactions in 15 seconds. 0.2 cc. (1: 500,000) of adrenalin; small nictitating reaction in 17 seconds; small pupil reaction in 25 seconds. While the pocket was still clipped off blood was obtained from the abdominal aorta.

Left adrenal weighed 0.206 gm. and contained 0.20 mg. of epinephrin; the right adrenal weighed 0.194 gm. and contained 0.18 mg. of epinephrin.

In this cat 3 days after transection of the cord between the fifth and sixth thoracic segments no eye reactions could be evoked by adrenal blood even with relatively long periods of collection in the cava pocket, periods which would certainly have given good reactions either with intact central nervous system or with the cord cut in the cervical region. The reactions were not lacking because the iris and nictitating membrane were incapable of responding to small quantities of epinephrin. For even at an advanced period in the experiment, after the withdrawal of several samples of adrenal blood through a cannula in the cava, good eye reactions were obtained on injection of 0.001 mg. of epinephrin, and quite detectable reactions on injection of 0.0004 mg. Tested with rabbit intestine and uterus segments, the adrenal blood gave a negative result (Fig. 7, Observation 27; Fig. 8, Observations 6 and 12), although a concentration of epinephrin of 1: 60,000,000 (Fig. 8, Observation 37) could easily have

been detected by the intestine. There was evidence that the adrenal blood (third specimen) could not have contained even 1: 100,000,000 epinephrin, corresponding to an output of at most 0.000003 mg. per minute, not one-hundredth of the output to be expected in a normal cat under the experimental conditions. It must be repeated that there was no evidence that any epinephrin was being discharged by the adrenals. Nothing could be more striking than the contrast between the concentration and output per minute of epinephrin in this cat and in those whose spinal cord was transected towards the lower level of the cervical region. For instance, the concentration in the second adrenal specimen in Experiment 5 was 1: 2,000,000; and the calculated output per minute, 0.0002 mg. Even when the cord was divided (Experiment 8) two segments higher in the dorsal region, through the third thoracic segment, although a slight epinephrin liberation was detected by intestine segments, the output was enormously reduced (to 0.000006 mg. per minute).

Experiment 8. Condensed Protocol.—Cat; weight 3.65 kilos. Left superior cervical ganglion excised 10 days before the experiment. Spinal cord transected between the third and fourth dorsal vertebræ 3 days before the experiment. Condition excellent. Ether anesthesia was used throughout the experiment. Long cava pocket prepared in the usual manner, renal, celiac, and mesenteric arteries, and abdominal aorta being tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
a.m.				
11.00		1 min., 20 sec.	None.	None.
11.05		1 " 50 "	"	"
11.10		3 "	"	"
11.15		5 "	"	"
11.30	Injected 0.5 cc. (1: 530,000) of adrenalin into jugular vein.		Excellent; in 10.6 sec.	Excellent; 10.6 sec.
11.35	Injected 0.2 cc. (1: 530,000) of adrenalin into jugular vein.		Very good; in 14.8 sec.	Very good; 14.8 sec.
11.38	Injected 0.5 cc. (1:2,000,000) of adrenalin into jugular vein.		Small; in 22 sec.	None.
11.40	Injected 0.5 cc. (1:2,000,000) of adrenalin into jugular vein.		Small; in 22 sec.	"

11.55 a.m. Obtained indifferent blood from the other jugular.

12.00 m. Inserted cannula in cava (short pocket) and collected adrenal blood as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	gm.		*gm.*
1	3.6	2 min.	1.8
2	8.8	6 "	1.46
3	5.0	6 "	0.83
4	5.5	7 " 30 sec.	0.73
5	4.0	6 "	0.66

While the pocket was still clipped off a second specimen of indifferent blood (jugular) was obtained. The autopsy showed that the spinal section (between the third and fourth dorsal vertebræ) was through the origins of the third pair of thoracic nerves. The bloods were tested on rabbit segments. The right adrenal weighed 0.200 gm. and contained 0.18 mg. of epinephrin; the left adrenal weighed 0.208 gm. and contained 0.18 mg. of epinephrin.

The concentration of epinephrin in the third adrenal specimen (Fig. 9, Observation 30) was distinctly less than 1: 135,000,000 (Observation 32), and very much less than 1: 70,000,000 (Observation 28). The concentration in the fifth adrenal specimen was somewhat greater than 1: 135,000,000. The eye reactions were negative, even when the cava pocket was closed for as much as 5 minutes, although the injection of 0.00025 mg. of epinephrin gave a definite pupil dilatation, and the injection of 0.0004 mg. a very good dilatation of the pupil and retraction of the nictitating membrane.

The results of the whole series of experiments are singularly consistent, particularly in view of the fact that there has been no selection of experiments. The eight experiments comprise all those performed, except one which has not been reported because the animal died before it was satisfactorily completed.[5] The results indicate

[5] In this animal the cord was divided between the second and third thoracic vertebræ, just below the origins of the second thoracic nerves, or nearly a segment higher than the highest dorsal section in the experiments reported. 3 days afterwards the eye reactions (the left superior cervical ganglion had been excised a week before the experiment) were found negative with collections in the cava pocket up to more than 3 minutes, although very good pupil and nictitating reactions were elicited by injection of 0.0015 mg. of epinephrin, and slight reactions by the injection of 0.0006 mg. The blood flow at the time of these observations was quite satisfactory, but the animal died be-

clearly that there exists in the cord between the last cervical segment and the fourth thoracic segment a mechanism which sustains the output of epinephrin from the adrenal glands after the cord is severed from the higher parts of the central nervous system. The experiments prove definitely that the center does not extend lower than the thoracic segment mentioned, and that at least an important part of it lies below the level of the last cervical segment. The possibility, however, is not excluded that the center may extend for some distance above the last cervical segment. It is of interest in connection with the currently accepted view of the development of the adrenal medulla, that the portion of the cord at which the sympathetic outflow begins should be identified as a center controlling the liberation of the only constituent of its secretion hitherto definitely recognized. If epinephrin in the quantities and concentrations in which it appears in the adrenal blood could be shown to fulfil an important office in maintaining the function of the sympathetic by activating certain of its elements, or by heightening or prolonging the effects resulting from its excitation, the location of an epinephrin center in the sympathetic region of the cord might perhaps acquire a new significance.[6] It might

fore adequate adrenal blood samples could be drawn off from the cannula in the cava. The small quantity of adrenal blood obtained while the blood was flowing very slowly showed a good concentration of epinephrin as tested on the rabbit segments. Since the eye reactions cannot in general detect outputs of epinephrin easily detectable by the rabbit segments, this result, although no great stress can be laid upon it in the absence of better samples of adrenal blood, is quite consistent with the general conclusion deduced from the other experiments as to the position and limits of the portion of the cord concerned in epinephrin secretion from the adrenals.

[6] It is difficult to demonstrate that the epinephrin spontaneously liberated from the adrenals has any effect upon the blood pressure unless its action is accumulated by collecting the adrenal blood in a cava pocket and then releasing it. The majority of recent observers have not seen any change in the blood pressure when the adrenal veins are carefully clipped. On the denervated eye, however, we have observed a phenomenon which indicates that even the small concentrations of epinephrin which can exist in the capillary blood when the adrenal blood is passing steadily into the circulation without being accumulated in the cava pocket can produce a demonstrable effect upon these extraordinarily sensitive objects. When the pupil has been dilated or the nictitating membrane retracted by release of adrenal blood collected in the cava pocket, the dilatation

then be permissible to speculate upon the possibility that the relative constancy of the epinephrin discharge, so puzzling on the hypothesis that it directly influences physiological events in virtue of the truly gross changes necessary to produce a detectable hyper- or hypoadrenalinemia, is associated with a more general and permanent action upon the sympathetic mechanisms, which does not entail the necessity of abrupt outbursts and remissions in the rate of liberation. To employ a simile which is doubtless excessively crude: if epinephrin is not the horse in the sympathetic machine, which must go now faster, now slower; nor even the whip which must sometimes be wielded vigorously and then be laid aside, is it not perhaps the lubricant which, whether the axle turns fast or slow, need not vary much in amount?

The possibility must, however, not be lost sight of, that epinephrin although the first definite constituent of the adrenal secretion to be discovered is not the only, nor the most important one which exists. It is difficult to conceive of a nervous control so complete as that which governs the output of epinephrin being developed in the case of a substance functionally unimportant. Yet, as we have shown in another place,[2] the output of epinephrin from the adrenals in cats is greatly and permanently reduced or abolished by section of the adrenal nerves without apparently interfering with the life or health of the animal. Section of the dorsal cord which, as has been shown above, produces a similar effect upon the output of epinephrin is also well known to be compatible with good health and long survival. Is there perhaps some as yet unknown substance of more importance than epinephrin which is normally given off from the adrenals under the influence of nerves, the secretion of which is eventually resumed after the nerves have been severed?

SUMMARY.

1. After section of the spinal cord in cats in the cervical region, as low as the last cervical segment, epinephrin continues to be liberated from the adrenal glands. This liberation has all the characters of

of the pupil disappears more slowly and the nictitating membrane comes forward more gradually when the pocket is left open than when it is clipped. Obviously, the steadily liberated epinephrin exerts an effect in prolonging the reactions once they have been elicited.

the normal secretion with intact central nervous system. It is sustained through the same nerve paths connecting the cord with the adrenals.

2. After section of the cord in the middorsal region the spontaneous liberation of epinephrin from the adrenals is abolished within the limits of detectability by the methods employed (denervated eye reactions of Meltzer, and rabbit intestine and uterus segments).[7]

3. The portion of the cord concerned in the liberation of epinephrin does not appear to extend much below the third thoracic segment.

4. In acute experiments on cats under urethane anesthesia no change in the rate of liberation of epinephrin, which could be detected by the tests employed, was observed when the cord was severed in the cervical region.

EXPLANATION OF PLATES.

PLATE 44.

FIG. 1. Intestine tracings. Blood from a cat. At 2 Ringer's solution was replaced by jugular blood, and this at 3 by the second adrenal blood specimen, collected with intact spinal cord. At 6 and 9 Ringer's solution was replaced by jugular blood, and this at 7 and 10 by the sixth and eighth adrenal blood specimens respectively, collected after section of the cord between the fourth and fifth cervical segments. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-third.

FIG. 2. Intestine tracings. Blood of the same cat used for Fig. 1. At 35 Ringer's solution was replaced by arterial blood, and this at 36 by the second adrenal blood specimen, collected before section of the cord. At 43 Ringer's solution was replaced by arterial blood, and this at 44 by the third adrenal blood specimen, collected just after transection of the cord between the fourth and fifth cervical segments. All the bloods were diluted with three volumes of Ringer's solution. At 37 Ringer's solution was replaced by arterial blood diluted with three volumes of Ringer's solution, and this at 38 by arterial blood to which adrenalin had been added to make up a concentration of 1: 13,000,000, the adrenalin blood being then diluted with three volumes of Ringer's solution. Reduced one-third.

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[7] We have since found that semisection of the cord (between the fourth and fifth dorsal segments in one cat, between the third and fourth segments in another) abolished the liberation of epinephrin from the adrenal of the same side without affecting the liberation from the other adrenal.

PLATE 45.

FIG. 3. Intestine and uterus tracings. Blood of the same cat used for Figs. 1 and 2. At 19 Ringer's solution was replaced by jugular blood, and this at 20 by the eighth adrenal blood sample, collected after section of the cord between the fourth and fifth cervical segments. Both bloods were diluted with eight volumes of Ringer's solution. At 21 and 23 Ringer's solution was replaced by adrenalin in jugular blood, made up to 1 : 1,600,000 and 1 : 800,000, respectively, and then diluted with eight volumes of Ringer's solution. 4, 5, and 6 are uterus tracings. At 4 Ringer's solution was replaced by the eighth adrenal blood specimen, at 5 by the second, and at 6 by indifferent (arterial) blood; all were diluted with seven volumes of Ringer's solution. Reduced one-third.

FIG. 4. Intestine tracings. Adrenal blood from a cat after section of the cord between the last cervical and first thoracic segments. At 18, 20, and 22 Ringer's solution was replaced by indifferent (arterial) blood, and this at 19, 21, and 23 by the fourth, second, and third adrenal specimens, respectively. All the bloods were diluted with two volumes of Ringer's solution. Reduced one-half.

FIG. 5. Intestine tracings showing some of the adrenalin assays for the adrenal bloods used for Fig. 4. At 26 and 28 Ringer's solution was replaced by indifferent (arterial) blood, diluted with two volumes of Ringer's solution and this at 27 and 29 by adrenalin in arterial blood, made up to 1: 1,500,000 and 1: 2,-500,000, respectively, and then diluted with two volumes of Ringer's solution. Reduced one-half.

PLATE 46.

FIG. 6. Uterus tracings. Blood of the same cat used for Figs. 4 and 5. At 36 Ringer's solution was replaced by the third adrenal specimen; at 37, by the second, both diluted with eight volumes of Ringer's solution. The third specimen has a stronger effect than the second, corresponding to the slower blood flow during its collection. But as the increase of tone produced by the second specimen even was nearly maximal, greater dilution was necessary to show the difference clearly. At 38 Ringer's solution was replaced by the second adrenal specimen, and at 39 by the third, both diluted with twelve volumes of Ringer's solution. The difference is now evident. At 40 Ringer's solution was replaced by the fourth, and at 41 by the third adrenal specimen, both diluted with sixteen volumes of Ringer's solution. The increase of tone produced by the third specimen was now so nearly maximal for the condition of the segment at the time that greater dilution was resorted to, to bring out the difference, clearly seen in Observations 43 and 44, where Ringer's solution was replaced by the third and fourth specimens respectively, both diluted with twenty-four volumes of Ringer's solution. At 42 Ringer's solution was replaced by indifferent (arterial) blood, diluted with twenty-four volumes of Ringer's solution. Reduced one-half.

FIG. 7. Intestine tracings. Adrenal blood from a cat after section of the cord between the fifth and sixth dorsal segments. At 25 Ringer's solution was replaced by indifferent (arterial) blood, and this at 27 by the third adrenal specimen, both bloods being undiluted. The magnification is high, nearly twice as great as in Fig. 8, in order to afford the best chance for an inhibitory effect to be seen on the tracing. The writing point went somewhat above the drum after the addition of the adrenal blood and no inhibition was produced. To save space, the tracing has been cut horizontally. To reconstruct it, the right-hand portion may be imagined to be pushed up to the top of the figure and then to the left till it is in line with the left-hand portion.

PLATE 47.

FIG. 8. Intestine tracings. Blood from the same cat as in Fig. 7, but with a smaller magnification. At 5 and 11 Ringer's solution was replaced by arterial blood, and this at 6 and 12 by the fourth and the third adrenal specimens, respectively, the bloods being diluted with four volumes of Ringer's solution. At 34 and 36 Ringer's solution was replaced by arterial blood (undiluted), and this at 35 and 37 by adrenalin in arterial blood (1: 40,000,000 and 1: 60,000,000 respectively). The adrenalin blood was undiluted. Reduced one-third.

FIG. 9. Intestine tracings. Adrenal blood from a cat after section of the cord through the third thoracic segment. At 29 Ringer's solution was replaced by jugular blood, and this at 30 by the third adrenal blood specimen. At 27 and 31 Ringer's solution was replaced by jugular blood, and this at 28 and 32 by jugular blood to which adrenalin had been added to make up a concentration of 1: 70,000,000 and 1: 135,000,000, respectively. All the bloods were undiluted. Reduced one-third.

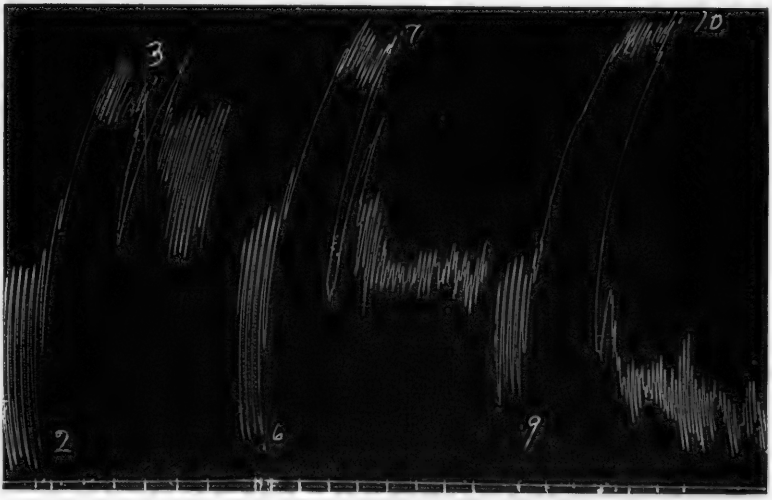

FIG. 1.

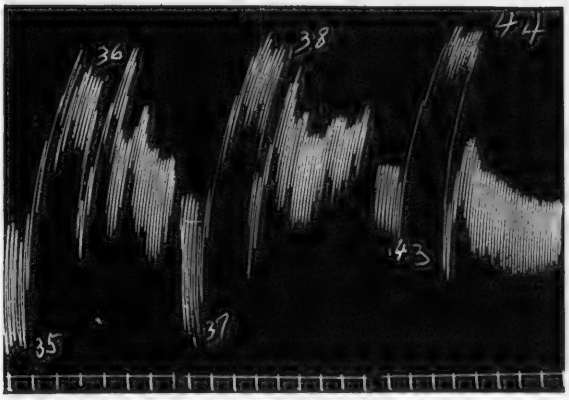

FIG. 2.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

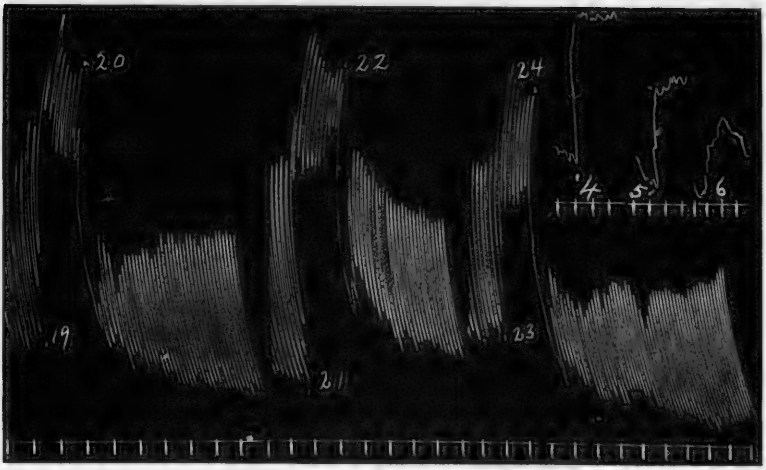

FIG. 3.

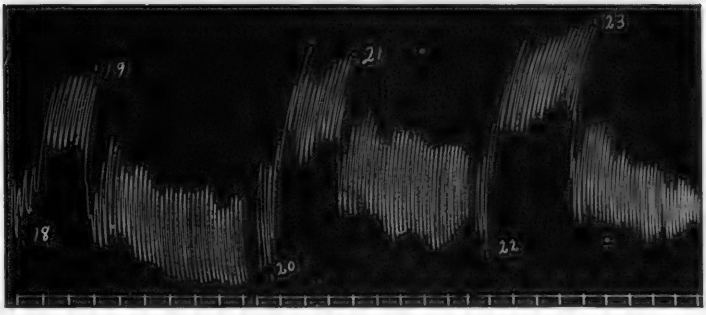

FIG. 4.

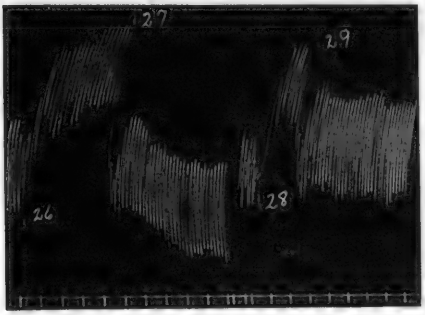

FIG. 5.

(Stewart and Rogoff: Spontaneous liberation of epinephr'n.)

FIG. 6.

FIG. 7.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

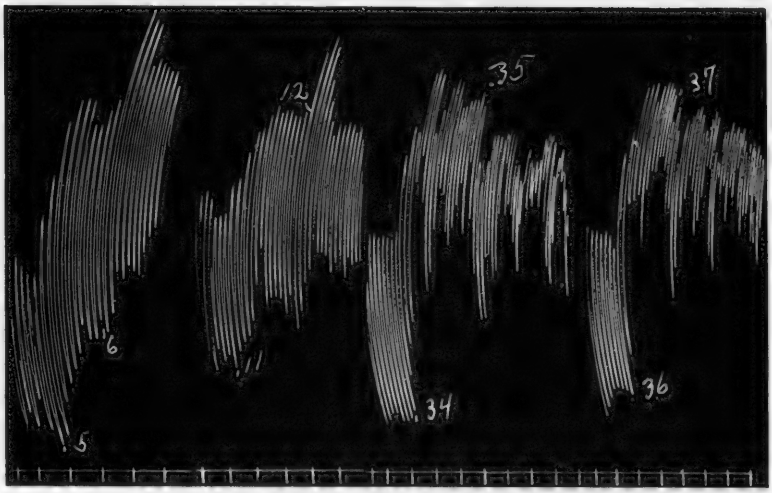

FIG. 8.

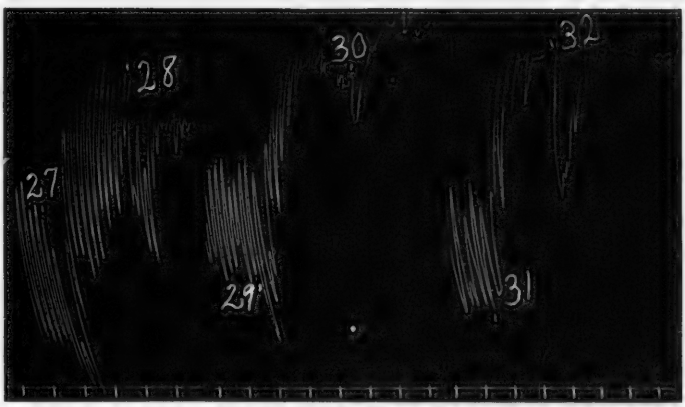

FIG. 9.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

EFFECT OF STIMULATION OF SENSORY NERVES UPON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS.

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Plates 48 to 53.

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The fact that the spontaneous liberation of epinephrin is dependent upon the integrity of certain efferent nerves running in the sympathetic system has led to attempts to influence the rate of liberation reflexly. We have previously[1] published experiments in which the rates of liberation of epinephrin during and without stimulation of brachial nerves (in the cat) were compared by means of the denervated eye reactions of Meltzer and by the rise of blood pressure produced when adrenal blood collected for a known time in a cava pocket is released. The results were negative.

We have since repeated the experiments on cats and dogs, drawing off blood from the cava pocket and testing it on rabbit intestine and uterus segments. In this way the adrenal blood can, of course, be applied to the test-objects without dilution if so desired, whereas, with the eye and blood pressure reactions, it is necessarily much diluted. On the other hand, the use of the latter reactions has some great advantages. The blood is not withdrawn from the vessels, and accordingly there is no danger of loss of a part of the epinephrin in the necessary manipulations before the blood is applied to the segments. The development of the pressor property of clotted blood, a serious complication for some of the methods of testing extravascular blood (frog perfusion, artery rings), is avoided. Possible effects upon the rate of liberation of the loss of blood, or even of the loss of the epinephrin in it when withdrawn from the body are also excluded. Most impor-

[1] Stewart, G. N., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916. viii, 517.

tant of all, the rise of blood pressure, especially if interpreted by the aid of the eye reactions, affords a direct quantitative comparison of the amount of epinephrin liberated in successive observations.

Technique.—The adrenal blood was collected through a boiled and oiled cannula in the inferior cava. The cava pocket was usually much shorter than that employed for the eye and blood pressure reactions, where it was essential to have as roomy a pocket as possible. With the short pocket only a small dead space is left filled with blood which has passed through the adrenals during one set of experimental conditions at the moment when the experimental conditions have been changed. To reduce still further any overlapping of the blood samples successively collected with and without stimulation, excitation of the sensory nerve was started slightly before completion of the collection of the preceding "no stimulation" sample, and *vice versa.* Once begun, the flow of blood from the cannula was interrupted, sample after sample being collected. The time of collection and the weight or volume of blood being accurately measured, the rate of blood flow during the collection of each sample is known. This is indispensable, of course, for estimating the rate of liberation of epinephrin by reactions which, like the segment tests, give only the concentration. Since, as we have found,[2] the concentration in successive samples tends to increase as the blood flow slackens, we varied the order of stimulation and no stimulation observations, interposing for example, a stimulation period between two no stimulations, and *vice versa.* Richards and Wood,[3] in their work on the influence of stimulation of the depressor upon suprarenal secretion, have recognized the necessity of measuring the rate of flow of the blood, and the advisability of not taking the samples in a uniform order. The stock adrenalin used for the epinephrin assays was always freshly assayed by the colorimetric method of Folin, Cannon, and Denis.[4]

In our previous observations we stimulated the brachial nerves, as it was convenient in forming the cava pocket to clamp the abdominal aorta, and the sciatic nerve was therefore not available. Since the upper thoracic portion of the spinal cord can sustain a substantial liberation of epinephrin after section of the cord in the cervical region,[5] the sciatic might perhaps be considered more likely to yield positive effects than the brachial. Elliott's conclusion[6] that after section of the brain stem just in front of the anterior corpora quadrigemina exhaustion of the epinephrin store of the adrenal by stimulation of sensory nerves occurs, while it does not take place if the cord has been cut just below the bulbar vasomotor center, does not, as already pointed out,[1] necessarily indicate that the center concerned in epinephrin liberation, on which Elliott made no experiments, is as

[2] Stewart and Rogoff, *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 77.
[3] Richards, A. N., and Wood, W. G., *Am. J. Physiol.*, 1915–16, xxxix, 54.
[4] Folin, O., Cannon, W. B., and Denis, W., *J. Biol. Chem.*, 1912–13, xiii, 477.
[5] Stewart and Rogoff, *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 143.
[6] Elliott, T. R., *J. Physiol.*, 1912, xliv, 374.

high as this. But if it were, brachial stimulation ought to hit it, as well as sciatic stimulation. To be sure, however, of stimulating afferent nerves favorably situated with reference to one or the other of the centers concerned, if there is more than one, we used both sciatic and brachial in these experiments.

While cats proved suitable for the previous observations in which blood was not withdrawn, it was judged advisable in the present series to use dogs also, in order to make certain of a good and uninterrupted flow of blood, which would completely wash out the pocket and cannula, and thus prevent overlapping of the successive samples.

Experiment 1. Condensed Protocol.—Dog; weight 10 kilos. Ether anesthesia. 45 cc. of blood withdrawn from jugular vein. Cava pocket tied off. Intestinal arteries and abdominal aorta not tied. Right iliac artery ligated and cannula inserted into the right iliac vein. The left iliac vein was not tied, and was clamped just before the collection of blood was begun, in order to spare the left sciatic as much as possible. The left sciatic and brachial nerves were prepared for stimulation. The following blood specimens were collected from the cannula in the iliac.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		*cc.*	
1	13.0	1 min., 40 sec.	8.0	None.
2	17.3	2 " 25 "	7.2	Brachial.
3	9.2	1 " 40 "	5.8	"
4	7.4	1 " 47 "	4.4	None.
5	10.2	3 " 10 "	3.2	Sciatic.
6	7.5	3 " 10 "	2.4	"
7	5.0	3 " 24 "	1.5	None.

While the pocket was still clipped off, 53 cc. of arterial blood were obtained from the carotid artery. Combined weight of adrenals 1.050 gm.

It will be seen from Figs. 1 to 3, that no difference could be made out between the inhibitory effects produced on the rabbit intestine segments by the various adrenal blood samples, which could be connected with the presence or absence of nerve stimulation. Thus in Fig. 1, the second sample collected during brachial stimulation (Observation 5) caused practically the same effect as the first sample, collected without stimulation, and the blood flows were about the same during collection of the two samples. The third sample, with nerve stimulation, gave a greater inhibition (Observation 7) than the first, without stimulation; but stimulation of the nerves had nothing

to do with this, since the nerves were also stimulated during collection of the second sample (Observation 5). The explanation of the greater inhibition produced by the third sample is that the blood flow was slower during its collection, and the rate of liberation of epinephrin per minute remaining the same, the concentration was necessarily greater. The adrenalin assays showed that the concentration in the first and second samples was somewhat more than 1: 3,300,000, corresponding to an output of epinephrin per minute of 0.0025 mg. (0.00025 mg. per kilo of body weight per minute), and that the third specimen was not twice as strong as the first or second. This is a normal output for a dog under the experimental conditions, as estimated in drawn adrenal blood on rabbit intestine segments.

In Fig. 2 it is shown that sciatic stimulation also produced no demonstrable effect, the slight preponderance of the fifth sample (Observation 15) as compared with the fourth (Observation 13) being plainly associated with the somewhat slower flow during collection of the latter. In Fig. 3 the sixth and seventh adrenal samples were compared with greater magnification; but the result was the same; the blood collected during stimulation of the sciatic caused no greater effect than that collected without stimulation. It is probable, however, that the concentration of epinephrin in the sixth and seventh samples was such as to produce the maximum inhibition of which the segment was capable at the time. That the sixth specimen really contained somewhat less epinephrin than the seventh, corresponding to the greater blood flow, is brought out by the uterus tests (Fig. 4, Observations 48 and 50). The same progressive increase in concentration in the successive samples shown by the intestine tracings is exhibited on the uterus tracings, only, as is commonly the case, even more sharply. The difference between the fourth and third specimens (Observations 44 and 45) would unquestionably have been brought out clearly with a greater degree of dilution, as the increase of uterus tone in these observations was already approaching, if it had not indeed reached the maximum for the segment.

Experiment 2. Condensed Protocol.—Cat; weight 2.9 kilos. Urethane anesthesia. Obtained specimen of jugular blood, then isolated left sciatic nerve and prepared it for stimulation. Cava pocket made, tying off intestinal and renal vessels and right iliac artery. Cannula inserted into the cava, making a short pocket. Adrenal blood was then collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	gm.		*gm.*	
1	1.5	1 min., 25 sec.	1.1	None.
2	4.4	4 "	1.1	Sciatic.
3	1.4	6 "	0.23	None.
4	1.8	9 " 30 "	0.19	"
5	2.3	13 "	0.18	Sciatic.
6	1.7	8 "	0.21	None.
7	2.2	10 "	0.22	"

During stimulation of the sciatic, the pupils dilated widely, the respiratory movements were increased, and there were reflex movements indicating that the stimulus was effective. Stimulation was begun ½ to 1 minute before collection of the corresponding specimen, and was stopped ½ to 1 minute before the collection of the specimen ceased, to allow for washing out of the dead space. The flow was notably diminished after the first period of strong stimulation; that is, after the collection of the second specimen. After the last adrenal sample was collected indifferent blood was obtained from the abdominal aorta. Combined weight of the adrenals 0.347 gm.

Specimens of the tracings with rabbit segments are given in Figs. 5 to 7. They show the same general result as in Experiment 1; that is, a progressive increase in epinephrin concentration in successive adrenal samples, unmodified within the limits of sensitiveness of the method by stimulation of the sciatic.

Experiment 3. Condensed Protocol.—Cat; weight 2.58 kilos. Urethane anesthesia. Cava pocket prepared with cannula in left renal vein. Renal and intestinal arteries tied, but not the abdominal aorta. Sciatic nerve prepared for stimulation. Adrenal blood samples were collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		*cc.*	
1	10.0	5 min.	2.0	None.
2	9.0	6 " 30 sec.	1.5–2.0*	Sciatic.
3	10.3	5 "	2.0	None.
4	10.0	—	—†	During asphyxia.

* Part of the specimen was lost by accidentally spilling from the container.

† The blood clotted in the cannula at the end of the collection, and was gradually slowing so that the flow per minute could not be properly calculated.

Indifferent blood was obtained from the abdominal aorta. Indifferent blood from the carotid of another cat was also used in the tests after testing it against the blood from the abdominal aorta, and finding it to have the same tone-increasing power. Combined weight of the adrenals 0.328 gm.

The same results were obtained with the intestine and uterus segments as in the preceding experiments; there was equality or a progressive increase of epinephrin in the successive adrenal blood samples according to whether the blood flow remained constant or slackened. The output of epinephrin per minute was not modified, within the limits of accuracy of the assays, by stimulation of the sciatic. The concentration of epinephrin in the second and third adrenal specimens was about the same (1: 3,500,000) corresponding to a liberation of 0.0006 mg. per minute (more than 0.0002 mg. per kilo of body weight per minute), a normal output as estimated on drawn adrenal blood by rabbit segments. The uterus segments could detect a concentration of 1: 24,000,000 adrenalin in indifferent blood as compared with the indifferent blood itself.

Experiment 4. Condensed Protocol.—Dog; weight 8.5 kilos. Urethane and ether anesthesia. Cava pocket prepared, tying off the renal and intestinal arteries. The abdominal aorta was not clamped till just before the collection of blood. Cannula in right iliac vein. Left sciatic prepared for stimulation. About 5 cc. of blood were first collected from the pocket and discarded so as to wash the pocket free of any epinephrin which might have been liberated in the manipulations. The following adrenal samples were then collected.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		*cc.*	
1	34.0	2 min., 30 sec.	13.6	Sciatic.
2	24.0	3 "	8.0	None.
3	22.0	3 "	7.3	"
4	25.0	4 "	6.2	Sciatic.

Serum was obtained from the bloods by centrifugalization. The corpuscle sediment was about one-third of the total volume.

In this experiment serum was used for the segment tests instead of blood, so as to increase the chance of detecting any difference due to stimulation, since serum contains a greater concentration of epi-

nephrin than the blood from which it is derived.[7] The result was the same as in the other experiments. Thus, the second adrenal specimen, collected without stimulation (Observation 15, Fig. 8) had a somewhat greater inhibitory effect upon the intestine segments than the first specimen collected during sciatic stimulation (Observation 11), corresponding to the difference in blood flow. The effect of the third specimen (Observation 6, Fig. 9) is greater than that of the second (Observation 4), and less than that of the fourth specimen (Observation 9), without apparent relation to the presence or absence of nerve stimulation. The adrenalin assay showed that the second specimen contained about 1:3,500,000 epinephrin, corresponding to 1:5,000,000 for the blood, an output per minute of 0.0016 mg. (0.0002 mg. per kilo of body weight per minute), a normal output for a dog, as estimated in this way. As this is a moderate concentration, and the first specimen contained still less, the failure of nerve stimulation to increase the concentration could not have been due to the initial concentration being near the possible maximum. The serum of the third specimen contained 1:3,000,000 epinephrin, corresponding to 1:4,300,000 for the blood. This gives the same output as for the second specimen (0.0017 mg. per minute), the concentrations being inversely proportional to the blood flows. The uterus tracings, some of which are reproduced in Fig. 10, confirm the conclusion that the first adrenal specimen (Observations 31 and 33) contained a smaller concentration of epinephrin than the second (Observations 32 and 34), although the sciatic had been stimulated during the collection of the first. Observation 30, Fig. 10, shows a much smaller effect with indifferent serum, compared with any of the adrenal sera, thus confirming the conclusion that the substance inhibiting the intestine was epinephrin.

The objection might be made that under the influence of the experimental conditions (anesthesia, trauma, etc.), the rate of liberation of epinephrin might be already so great that it could not be augmented by stimulation of afferent nerves. This objection has already been partly met by the fact that with moderate concentrations of epinephrin, as shown by adrenalin assays, nerve stimulation fails to

[7] Stewart and Rogoff, *J. Pharm. and Exp. Therap.*, 1916–17, ix, 393.

increase the concentration. There is no evidence that anesthetics increase the liberation demonstrably. In cats, some days after section of the spinal cord in the cervical region, we have found[4] that blood collected from the adrenal veins through a cannula in the inferior cava contains concentrations of epinephrin within the ordinary range, despite the fact that on account of the complete anesthesia below the level of the cord section it was not necessary to give an anesthetic. Elliott's result, that anesthetics cause diminution of the epinephrin store of the adrenals, is no proof, even if we admit that the diminution is really due in some direct way to the anesthetic, that the output of epinephrin is increased under their influence; since a diminution in the rate of formation of epinephrin would equally be accompanied by a diminution in the store.

Nevertheless, we made a number of experiments in which the animal was rendered insensitive by destruction of the cerebral cortex, or by increase of intracranial pressure without the use of anesthetics, except for a few minutes while the brain was being destroyed or the skull trephined for the insertion of the pressure bag.

Experiment 5. Condensed Protocol.—Dog; weight 7.6 kilos. Animal rendered insensible by destruction of the cerebral cortex with much of the underlying centrum ovale. It was shown at autopsy that none of the brain tissue behind the anterior edge of the anterior corpora quadrigemina had been destroyed. Ether was given only during destruction of the brain. Indifferent blood was obtained from the jugular vein. Cava pocket made. Right iliac artery, and intestinal and renal arteries tied off. Left sciatic nerve prepared for stimulation. Adrenal blood specimens collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	gm.		*gm.*	
1	3.5	1 min.	3.5	None.
2	7.0	3 "	2.3	"
3	7.7	4 " 30 sec.	1.7	Sciatic.

A fourth adrenal specimen was obtained without stimulation, but clotting in the cannula prevented accurate measurement of the time (3 gm. in 4 to 5 minutes). Capacity of the cava pocket, which was a long pocket, 0.9 to 1.0 gm. of blood.

In this animal no evidence was forthcoming, any more than in the others, that stimulation of the sciatic was associated with a detectable increase in the rate of epinephrin output. A few of the tracings are reproduced in Fig. 11. The second adrenal specimen, collected without stimulation (Observation 23), caused about the same amount of inhibition of the intestine as the third specimen, collected during sciatic stimulation (Observation 25). If anything, the effect of the second was somewhat greater than that of the third specimen. The flows were not very different for the two specimens. The fourth adrenal specimen (Observation 19), collected without sciatic stimulation, produced a decidedly greater effect than the third, corresponding to the much slower flow. Here is an instance where a nerve stimulation period between two periods without stimulation ought to have shown some change of concentration, as compared either with the preceding or the succeeding period, had the nerve stimulation been capable of evoking such a change. The first adrenal specimen, without nerve stimulation (Observation 21), has a greater inhibitory effect than either the second or third. But this is doubtless due to epinephrin liberated during manipulations while the pocket was being tied off. Although it is often possible to complete the operation without any evidence of manipulative discharge, yet, in order to be sure of avoiding errors due to this cause, the first specimen was always considered suspect, if it gave a higher concentration than the second, and in that case rejected. It was, in fact, collected separately for the purpose of insuring that the succeeding specimens should not contain any epinephrin liberated by massage, etc., during the formation of the pocket.

Uterus tracings confirmed the conclusion that the second and third adrenal specimens (Experiment 5) had about the same concentration of epinephrin, and that the fourth had a greater concentration than either the second or third. Adrenalin assays showed that the concentration in the third specimen was about 1: 3,000,000, corresponding to an output of 0.0006 mg. of epinephrin per minute (about 0.0001 mg. per kilo of body weight per minute). This is rather below than above the average output in anesthetized dogs, as estimated by rabbit segments.

Elliott has stated that brain injuries such as destruction of the cerebral hemispheres cause discharge of the epinephrin store of the adrenals. Although for the reason already mentioned, this would not of itself be sufficient proof that the rate of liberation of epinephrin is sensibly increased by the brain irritation, an experiment was made in which the animal was rendered insensitive by increasing the intracranial pressure, without any brain mutilation, by a thin rubber bag introduced through a trephine hole.

Experiment 6. Condensed Protocol.—Dog; weight 9.4 kilos. Under ether anesthesia the skull was trephined, and a rubber bag inserted. The bag was connected with a mercury manometer. The pressure in it was increased to 250 mm. of mercury, and the ether discontinued. Artificial respiration was started, although the dog was still breathing well spontaneously. A short cava pocket was made. The abdominal aorta was tied off below the renals, and the renal vessels were tied. Brachial nerves on one side prepared for stimulation. As the blood pressure fell the intracranial pressure was diminished. Adrenal blood was collected as follows: The first specimen (5 to 6 gm.) was rejected to avoid any epinephrin liberated during manipulation.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	gm.		*gm.*	
2	7.2	45 sec.	9.0	None.
3	12.0	1 min., 35 "	8.0	Brachial.
4	12.5	2 " 20 "	5.4	None.
5	9.2	2 " 30 "	3.7	Brachial.

Combined weight of adrenals 1.20 gm.

In Fig. 12 are reproduced a few of the intestine segment tracings from Experiment 6. They show that the third adrenal specimen, collected during brachial stimulation (Observation 31), while causing a somewhat greater inhibition than the second specimen, collected without stimulation (Observation 29), does not produce as great an inhibitory effect as the fourth specimen, collected without stimulation. The fifth adrenal specimen, collected during brachial stimulation (Observation 35), causes an inhibition not conspicuously different from that caused by the fourth specimen. As before, the progressive increase in epinephrin concentration associated with the gradual de-

cline in the rate of blood flow has not been sensibly altered by the nerve stimulation. This result was confirmed by the uterus tracings, some of which are reproduced in Fig. 13. If the third adrenal specimen (Observation 52) were compared merely with the second (Observation 51), it might be thought that the greater effect on the uterus produced by the third specimen indicated an increased output of epinephrin due to stimulation of the nerve. This conclusion is at once seen to be erroneous when we compare the effect of the fourth specimen (Observation 54) with that of the third, for the fourth is as much stronger than the third as the third is stronger than the second, and the nerves were not being stimulated during collection of the fourth specimen. To show the uniformity of the tracings, Observation 52 on the third specimen was interposed between two observations (51 and 53) on the second. In Observations 56 to 59, the second to fifth specimens were compared in a greater dilution, and just as in the case of the intestine tracings no such preponderance of effect was revealed in the third and fifth specimens as would be expected if the nerve stimulation during their collection had sensibly increased the output of epinephrin.

Adrenalin assays were made on the intestine, and in this case also on the uterus segments. The intestine segments are more generally useful than the uterus for assaying the concentration of epinephrin, although for bringing out qualitative differences the uterus is frequently much more sensitive than the intestine. But with favorable uterus segments good quantitative results are also obtained. The uterus from adult or nearly adult virgin rabbits is the best in our experience for all such work. The assays indicated that the second adrenal specimen contained about 1:9,000,000 epinephrin (Fig. 13, Observations 61, 64, 65, and 66); and the third specimen more than 1:9,000,000 but much less than 1:6,000,000 (Fig. 13, Observation 68), probably not far from 1:8,000,000. The fourth specimen had a greater concentration than 1:8,000,000, though distinctly less than 1:5,000,000, and somewhat less than 1:6,000,000, probably about 1:6,500,000. If the blood flows in the protocol are compared with these concentrations, it will be seen that the concentrations are approximately in the inverse ratio of the flows. In other words, during the collection of these adrenal samples, the output of epinephrin per minute (0.001 mg. or more

than 0.0001 mg. per kilo of body weight per minute) remained approximately constant, and was not sensibly modified by stimulation of the brachial nerve.

It did not seem probable that if negative results were yielded by unmixed adrenal vein blood, positive results would be obtainable with cava blood collected by a catheter anterior to the adrenal veins.[8] However, as the necessary operation for the catheter method is less severe than for the cava pocket method, the possibility could not be overlooked that the output of epinephrin in the periods without nerve stimulation might be less when extensive trauma was avoided. If this were so, an increase in the liberation produced by the stimulation of nerves might more readily make itself felt. Of course, it is impossible by this method to take account of any changes in the rate of blood flow through the adrenals; and comparisons of the concentration of epinephrin in the blood are only valid for the estimation of changes in the rate of liberation if alterations in the rate of blood flow are known, unless the assumption can be made that the blood flow remains constant during the whole experimental period. Nevertheless, the conditions which in the abdominal operation lead to notable alterations in the rate of blood flow were not so likely to be present with the catheter method, except in so far as the catheter itself might interfere with the flow of blood in the cava, and therefore we made a number of experiments in this way. Experiment 7 is an example.

Experiment 7. Condensed Protocol.—Cat; weight 2.875 kilos.

10.00 a.m. 5 gm. urethane by stomach tube.

11.00 a.m. Exposed left femoral vein and prepared it for catheter; prepared right sciatic nerve for stimulation.

11.20 a.m. Blood I obtained through catheter inserted to a level just anterior to adrenal veins. At autopsy, the eye of the catheter was found to be about 5 to 6 mm. anterior to the orifice of the right adrenal vein when inserted to the distance used in the experiment.

11.32 a.m. Started stimulation of sciatic.

11.37 a.m. Blood II collected through catheter at the same level, sciatic stimulation being continued throughout the collection.

11.40 a.m. Blood III obtained from lower cava through catheter, which was withdrawn 9 cm.

[8] Cannon, W. B., and Hoskins, R. G., *Am. J. Physiol.*, 1911–12, xxix, 274.

12.00 m. Blood IV obtained from catheter in the same manner as Blood I.

12.05 p.m. Blood V obtained from catheter anterior to the adrenal veins, collected during asphyxia.

12.09 p.m. Blood VI obtained from catheter lower down in cava, as for blood III.

After obtaining each of the bloods I, III, and IV, the catheter was withdrawn, cleaned, and oiled again. The bloods were withdrawn as uniformly as possible by the aid of an aspirator.

The result was negative. No difference was found by rabbit intestine and uterus segment tests between blood withdrawn without stimulation of the sciatic, and blood withdrawn during stimulation. None of the samples caused any inhibition of the intestine segments. We do not doubt that under favorable conditions (especially sensitive segments, slow blood flow in the inferior cava, and possibly a fortunate location of the eye of the catheter with reference to the adrenal vein orifices) samples of blood may sometimes be drawn from the inferior cava containing a sufficient concentration of epinephrin to yield distinct reactions. We failed to obtain such reactions because the epinephrin given off by the adrenals in the usual amount was too highly diluted by the cava blood. To illustrate the effect of this dilution we made some experiments, in which pure adrenal blood from the cava pocket and catheter blood from above the level of the adrenals, obtained from the same animal, were compared. In some observations catheter blood from the level of the adrenals collected while the adrenal veins were clipped was compared with blood from the same level collected through the catheter with the adrenal veins open, also with a negative result. Experiment 8 is an example of these experiments.

Experiment 8. Condensed Protocol.—Cat; weight 2.68 kilos. Urethane 3 gm. by stomach tube, and later on, 2 gm. more. Both adrenal veins isolated and prepared for clipping. Femoral vein prepared for catheter insertion. Blood I, drawn through catheter from level just anterior to adrenal veins; blood II, from catheter at the same level, but with adrenal veins clipped; blood III, obtained in same manner as blood I; blood IV (10 cc. in 11 minutes) obtained from cava pocket through cannula in right renal vein. After releasing the pocket by removing the clamps, blood V was obtained through the catheter in the same way as blood I. Indifferent (arterial) blood was obtained from the abdominal aorta. The catheter was withdrawn, cleaned, and oiled after each specimen was collected.

In Fig. 14 it is seen that catheter blood from the level of the adrenals displacing arterial blood (Observation 10) produced no inhibition of the intestine segment, although pure adrenal vein blood (Observation 8) caused good inhibition. Adrenalin assays showed that the concentration of epinephrin in the adrenal blood was not far from 1: 3,000,000. It was confirmed on uterus segments (Fig. 15, Observations 33,35, and 45) that the catheter blood caused no greater effect than indifferent blood, and much less than adrenal blood (Observations 34 and 36). The uterus segment, as it happened, gave practically no increase of tone with indifferent blood, which rendered the demonstration of the absence of detectable epinephrin in the catheter blood all the more convincing. Catheter blood collected with clipped adrenal veins (Observation 37) behaved in no respect differently from blood similarly collected, but with the adrenal veins open (Observation 39). The segment could easily detect epinephrin in the concentration of 1: 65,000,000 (Observation 46). From the relatively considerable increase of tone given by blood with this concentration of adrenalin, there is no doubt that a much smaller concentration could have been detected. Accordingly, the adrenal vein blood must have been diluted in the inferior cava much more than twenty times.

In Fig. 16 (Observation 20) catheter blood from the level of the adrenals, collected with the adrenal veins clipped, was displaced by catheter blood collected with the veins open. No change in the intestine segment curve was produced; that is, the adrenal blood was so much diluted in the cava that a sample of cava blood containing the adrenal contribution could not be discriminated by this intestinal segment from a sample of cava blood which could not have been mixed with any adrenal blood. Nor could it be discriminated from the indifferent (arterial) blood, since its replacement by this (Observation 21) left the curve unaffected. A prompt and marked inhibition was produced, however, when the arterial blood was in its turn displaced by the pure adrenal blood (Observation 22).

In the last experiment to be quoted (Experiment 9), blood was collected by a catheter at the level of the adrenals during stimulation of the sciatic and without sciatic stimulation. Pure adrenal blood was also collected from the same dog, during and without stimulation of the sciatic.

Experiment 9. Condensed Protocol.—Dog; weight 14.9 kilos. Ether anesthesia. A specimen of indifferent blood was collected from the jugular vein. Then the left femoral vein was prepared for insertion of the catheter, and the right sciatic nerve prepared for stimulation. The catheter was now inserted to a level just anterior to the adrenal veins. At autopsy it was verified, as in all the other experiments of this type, that the eye of the catheter was anterior to the orifices of the adrenal veins. Three samples of blood were now collected through the catheter, the first without sciatic stimulation (13.0 cc. in 1 minute and 30 seconds), the second during sciatic stimulation (13.8 cc. in 1 minute and 56 seconds), and the third during sciatic stimulation (12.6 cc. in 1 minute and 18 seconds). The catheter was now removed, washed, oiled again, and reinserted to the same level after an interval of 6 minutes; and a fourth specimen collected through the catheter without sciatic stimulation (23.5 cc. in 3 minutes and 12 seconds). A cava pocket was now made, the renal and left iliac vessels being tied, and a cannula inserted in the left iliac vein. The abdominal aorta was clamped at the bifurcation just before beginning the collection of blood from the pocket. Adrenal blood samples were obtained from the pocket as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		*cc.*	
1	15.2	1 min., 50 sec.	8.4	None.
2	16.1	2 " 5 "	8.0	Sciatic.
3	18.3	2 " 32 "	7.0	"
4	20.0	2 " 30 "	8.0	None.
5	22.9	3 " 55 "	5.7	"
6	11.4	3 " 45 "	3.0	Sciatic.
7	13.1	4 " 5 "	3.2	"
8	7.1	4 "	1.8	None.

Combined weight of adrenals, 1.15 gm.

The bloods were carefully compared on intestine and uterus segments and the degree of dilution of the adrenal blood with indifferent blood, which just caused the inhibitory effect on the intestine segments to become too slight to be detected with certainty, was determined. In Fig. 17 some of the tracings are given. At 8 the fifth adrenal blood replaced indifferent blood, causing a marked inhibition of the intestine segment. The concentration of epinephrin in this adrenal sample was assayed at about 1: 3,000,000, corresponding to an output of 0.002 mg. of epinephrin per minute (0.00013 mg. per kilo of body weight per minute). In the eighth specimen the concen-

tration was assayed at 1:1,100,000, about three times as great as in the fifth sample. This is approximately the inverse ratio of the blood flows, the rate of output of epinephrin being practically unchanged. At 24 blood collected with a catheter at the level of the adrenals during sciatic stimulation replaced indifferent blood. It produced no inhibition, but instead a further increase of tone. Observations 26 and 28 represent the effect of the fifth adrenal blood specimen diluted respectively with ten and with twenty volumes of indifferent blood. Inhibition of the segment can still be detected in each case. Diluted with forty volumes of indifferent blood (Observation 30) the fifth adrenal specimen produced an inhibition so slight, if any, that it could not have been detected unless perhaps by comparison with the preceding and succeeding observations. With this dilution the concentration of epinephrin in the blood was already only 1:120,000,000, and this blood was again diluted with four volumes of Ringer's solution before being applied to the segment. At 32, the adrenal blood, diluted with eighty volumes of indifferent blood, replaced the indifferent blood. No inhibition could be clearly detected any more than with the catheter blood (Observation 24).

We fail to see how it is possible to make exact experiments on the rate of liberation of epinephrin by the catheter method.

SUMMARY.

An attempt was made to determine whether stimulation of afferent nerves (sciatic and brachial) produced a detectable increase in the rate of liberation of epinephrin from the adrenals, as determined by testing adrenal vein blood on rabbit intestine and uterus segments. The result was negative.

EXPLANATION OF PLATES.

In all the tracings time is marked in half minutes.

PLATE 48.

FIG. 1. Intestine tracings. Blood of a dog anesthetized with ether. At 2 Ringer's solution was replaced by jugular blood, and this at 3 by the first adrenal blood specimen, collected without stimulation of nerves. At 4 Ringer's solution was replaced by jugular blood, and this at 5 by the second adrenal blood specimen,

collected during stimulation of the brachial. At 6 Ringer's solution was replaced by jugular blood, and this at 7 by the third adrenal blood specimen, collected during brachial stimulation. Reduced one-third.

FIG. 2. Intestine tracings. Blood of the same dog as in Fig. 1. At 12 Ringer's solution was replaced by jugular blood, and this at 13 by the fourth adrenal specimen, collected without stimulation of nerves. At 14 Ringer's solution was replaced by jugular blood, and this at 15 by the fifth adrenal blood specimen, collected during sciatic stimulation. At 19 Ringer's solution was replaced by jugular blood, and this at 20 by the second adrenal specimen, collected during brachial stimulation. At 21 Ringer's solution was replaced by jugular blood, and this at 22 by the first adrenal specimen, collected without stimulation of nerves. Reduced one-third.

FIG. 3. Intestine tracings. Blood of the same dog as in Figs. 1 and 2. Greater magnification. At 24 Ringer's solution was replaced by jugular blood and this at 25 by the sixth adrenal specimen, collected during sciatic stimulation. At 27 Ringer's solution was replaced by jugular blood, and this at 28 by the seventh adrenal specimen, collected without stimulation of nerves. Reduced one-third.

PLATE 49.

FIG. 4. Uterus tracings. Blood of the same dog as in Figs. 1 to 3. At 41 Ringer's solution was replaced by arterial blood; at 42, by the first adrenal specimen, collected without nerve stimulation; at 43, by the second adrenal specimen, collected during brachial stimulation; at 44, by the third adrenal specimen, collected during brachial stimulation; at 45, by the fourth adrenal specimen, collected without nerve stimulation. In Observations 41 to 45 the bloods were diluted with four volumes of Ringer's solution. At 47 Ringer's solution was replaced by the fifth adrenal specimen, collected during sciatic stimulation; at 48, by the sixth adrenal specimen, collected during sciatic stimulation; at 50, by the seventh adrenal specimen, collected without nerve stimulation. In Observations 47 to 50 the bloods were diluted with nine volumes of Ringer's solution. Reduced one-half.

FIG. 5. Intestine tracings. Blood from a cat anesthetized with urethane. At 4 Ringer's solution was replaced by jugular blood, and this at 5 by the second adrenal blood specimen, collected during sciatic stimulation. At 6 Ringer's solution was replaced by jugular blood, and this at 7 by the fourth adrenal specimen, collected without sciatic stimulation. The bloods were diluted with four volumes of Ringer's solution. Reduced one-third.

FIG. 6. Intestine tracings. Blood from the same cat used for Fig. 5. At 12 Ringer's solution was replaced by jugular blood, and this at 13 by the seventh adrenal specimen, collected without stimulation of the sciatic. At 14 Ringer's solution was replaced by jugular blood, and this at 15 by the fifth adrenal specimen, collected during sciatic stimulation. The bloods were diluted with two volumes of Ringer's solution. Reduced one-third.

FIG. 7. Uterus tracings. Blood of the same cat used for Figs. 5 and 6. At 28 Ringer's solution was replaced by jugular blood; at 29, by the second adrenal blood specimen, collected during sciatic stimulation; at 30, by the fourth adrenal specimen, collected without stimulation; at 31, by the fifth adrenal specimen, collected during sciatic stimulation; at 32, by the seventh adrenal specimen, collected without stimulation. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-half.

PLATE 50.

FIG. 8. Intestine tracings. Sera of a dog anesthetized with urethane and ether. At 10 Ringer's solution was replaced by serum of arterial blood, and this at 11 by serum of the first adrenal specimen, collected during sciatic stimulation. At 14 Ringer's solution was replaced by serum of arterial blood, and this at 15 by serum of the second adrenal specimen, collected without sciatic stimulation. Reduced one-third.

FIG. 9. Intestine tracings. Sera of the same dog as in Fig. 8. At 3 Ringer's solution was replaced by serum of arterial blood, and this at 4 by serum of the second adrenal specimen, collected without nerve stimulation. At 5 Ringer's solution was replaced by serum of arterial blood, and this at 6 by serum of the third adrenal specimen, collected without nerve stimulation. At 8 Ringer's solution was replaced by serum of arterial blood, and this at 9 by serum of the fourth adrenal specimen, collected during sciatic stimulation. Reduced one-third.

FIG. 10. Uterus tracing. Sera of the same dog used for Figs. 8 and 9. At 30 Ringer's solution was replaced by serum of arterial blood; at 31, by serum of the first adrenal specimen, collected during sciatic stimulation; at 32, by serum of the second adrenal specimen, collected without sciatic stimulation. The serum in Observation 30 was undiluted, in Observations 31 and 32 it was diluted with an equal volume of Ringer's solution. At 33 Ringer's solution was replaced by serum of the first adrenal specimen diluted with three volumes of Ringer's solution, and at 34 by serum of the second adrenal specimen similarly diluted. Reduced one-half.

PLATE 51.

FIG. 11. Intestine tracings. Blood of a dog rendered insensitive by destruction of the cerebral cortex. At 18 Ringer's solution was replaced by jugular blood, and this at 19 by the fourth adrenal specimen, collected without nerve stimulation. At 20 Ringer's solution was replaced by jugular blood, and this at 21 by the first adrenal specimen, collected without nerve stimulation. At 22 Ringer's solution was replaced by jugular blood, and this at 23 by the second adrenal specimen, collected without nerve stimulation. At 24 Ringer's solution was replaced by jugular blood, and this at 25 by the third adrenal specimen, collected during sciatic stimulation. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-half.

FIG. 12. Intestine tracings. Bloods from a dog rendered insensitive by increased intracranial pressure. At 28 Ringer's solution was replaced by indifferent (arterial) blood, and this at 29 by the second adrenal specimen, collected without brachial stimulation. At 30 Ringer's solution was replaced by arterial blood, and this at 31 by the third adrenal specimen, collected with brachial stimulation. At 32 Ringer's solution was replaced by indifferent blood, and this at 33 by the fourth adrenal specimen, without brachial stimulation. At 34 Ringer's solution was replaced by arterial blood, and this at 35 by the fifth adrenal specimen, collected with brachial stimulation. All the bloods were diluted with two volumes of Ringer's solution. Reduced one-third.

PLATE 52.

FIG. 13. Uterus tracings. Bloods from the same dog used in Fig. 12. At 50 Ringer's solution was replaced by indifferent (arterial) blood; at 51, by the second adrenal specimen, collected without brachial stimulation; at 52, by the third adrenal specimen, collected with brachial stimulation; at 53, by the second adrenal specimen; at 54, by the fourth adrenal specimen, collected without brachial stimulation. All these bloods were diluted with two volumes of Ringer's solution. At 56 Ringer's solution was replaced by the fourth adrenal specimen; at 57, by the third adrenal specimen; at 58, by the second adrenal specimen; at 59, by the fifth adrenal specimen, collected with brachial stimulation. These bloods were diluted with four volumes of Ringer's solution. At 61 Ringer's solution was replaced by indifferent (arterial) blood made up with adrenalin to a concentration of 1:8,000,000; at 66, by indifferent blood made up with adrenalin to a concentration of 1:9,000,000; at 68, by the indifferent blood with adrenalin to a concentration of 1:6,000,000. All the adrenalin bloods were diluted with four volumes of Ringer's solution before application to the segment. At 64 Ringer's solution was replaced by the second adrenal specimen, at 65 by the third adrenal specimen, each diluted with four volumes of Ringer's solution. Reduced one-half.

FIG. 14. Intestine tracings. Blood from a cat anesthetized with urethane. At 7 Ringer's solution was replaced by indifferent (arterial) blood, and this at 8 by adrenal blood. At 9 Ringer's solution was replaced by arterial blood, and this at 10 by catheter blood collected at the level of the adrenals. Reduced one-half.

FIG. 15. Uterus tracings. Blood from the same cat used for Fig 14. At 33 Ringer's solution was replaced by catheter blood from the adrenal level, and this at 34 by adrenal blood; at 35 Ringer's solution was replaced by catheter blood, and this at 36 by adrenal blood. At 37 Ringer's solution was replaced by catheter blood collected during the clipping of both adrenals, and this at 38 by adrenalin (1:1,000,000) in the same blood. At 39 Ringer's solution was replaced by catheter blood from the level of the adrenals, and this at 40 by adrenalin (1:3,300,000) in the same blood. At 42 Ringer's solution was replaced by

adrenalin (1: 16,500,000) in catheter blood; at 43, by the same catheter blood without the addition of adrenalin; at 45, by another catheter specimen collected at the adrenal level; at 46, by adrenalin (1: 65,000,000) in the catheter blood used for Observation 45.

PLATE 53.

FIG. 16. Intestine tracings. Blood from the same cat used for Figs. 14 and 15. At 19 Ringer's solution was replaced by catheter blood collected with the adrenal veins clipped, and this at 20 by catheter blood collected at the adrenal level with the adrenal veins open. At 21 this catheter blood was replaced by arterial blood, and this at 22 by adrenal blood. Reduced one-third.

FIG. 17. Intestine tracings. Bloods from a dog anesthetized with ether. At 7 Ringer's solution was replaced by indifferent blood, and this at 8 by the fifth adrenal specimen. At 23 Ringer's solution was replaced by indifferent blood, and this at 24 by catheter blood, collected at the level of the adrenals during sciatic stimulation. At 25 Ringer's solution was replaced by indifferent blood, and this at 26 by the fifth adrenal specimen, diluted with ten volumes of indifferent blood. At 27, 29, and 31 Ringer's solution was replaced by indifferent blood, and this at 28, 30, and 32 by the fifth adrenal specimen, diluted respectively with twenty, forty, and eighty volumes of indifferent blood. All the bloods were diluted with four volumes of Ringer's solution before application to the segment. Reduced one-third.

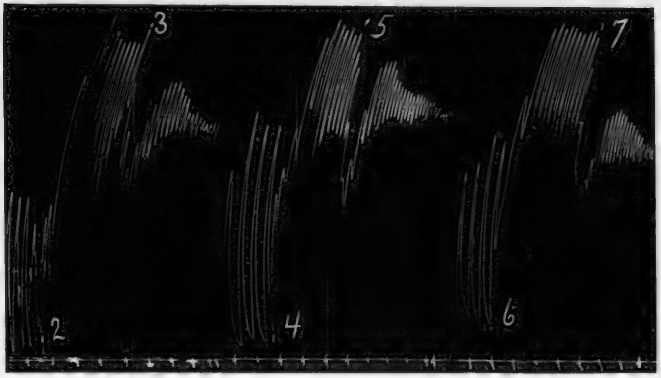

FIG. 1.

FIG. 2.

FIG. 3.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

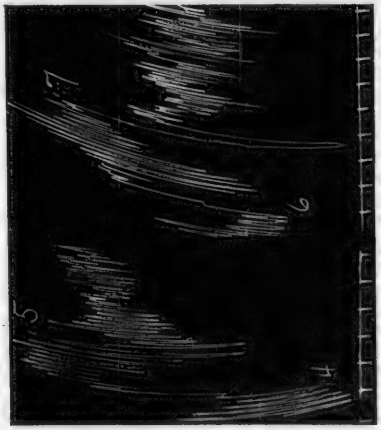

FIG. 5.

FIG. 7.

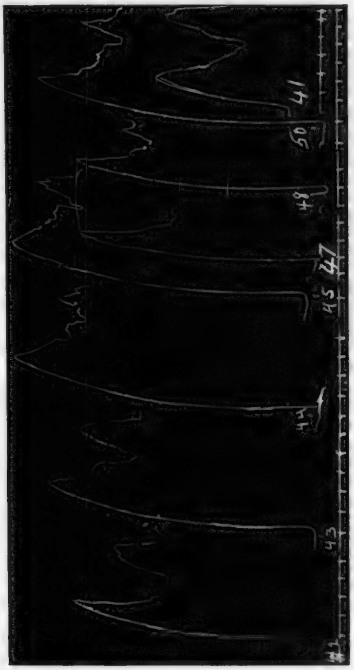

FIG. 4.

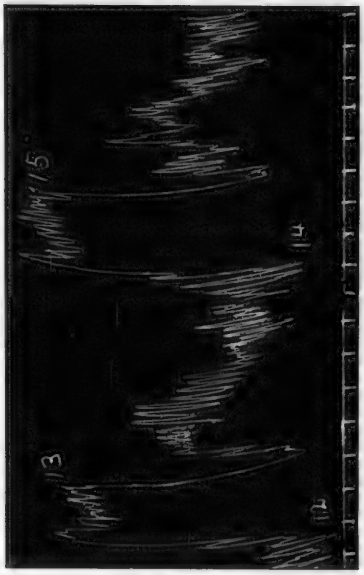

FIG. 6.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

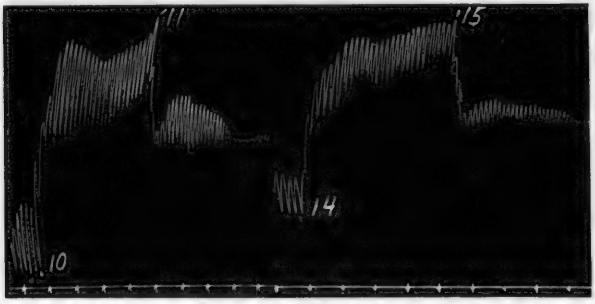

FIG. 8.

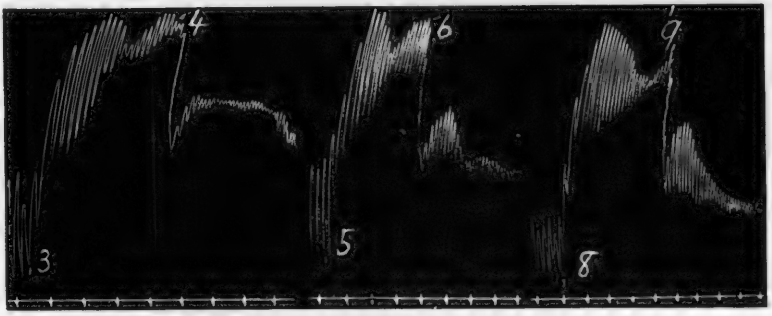

FIG. 9.

FIG. 10.

(Stewart and Rogoff: Rate of liberation of epinephrin.

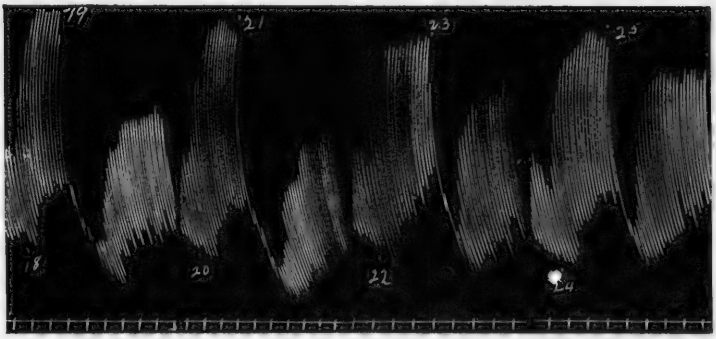

FIG. 11.

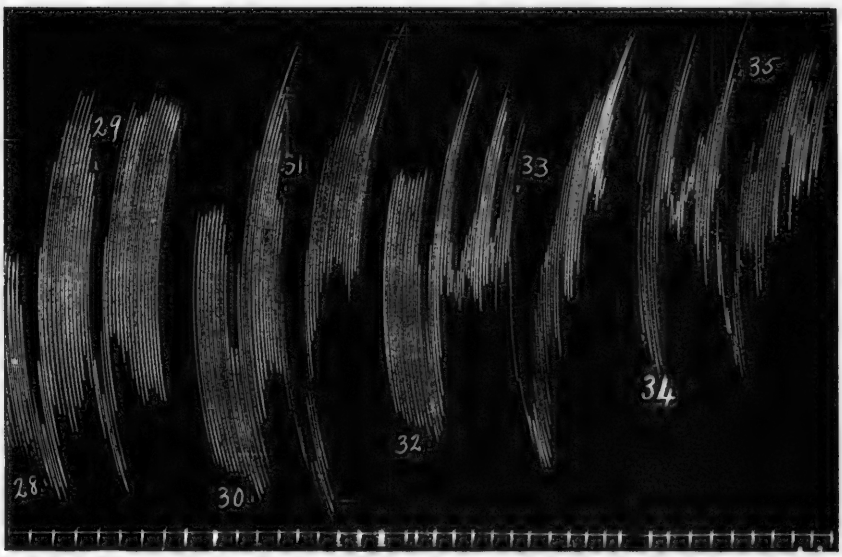

FIG. 12.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

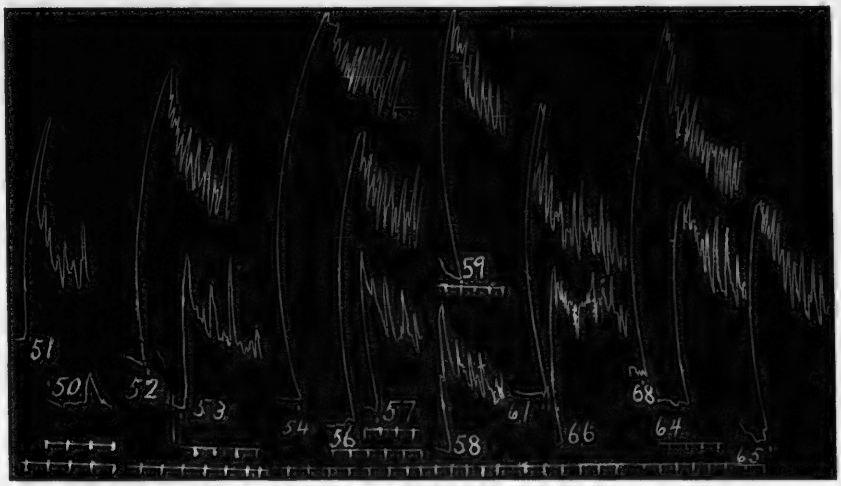

FIG. 13.

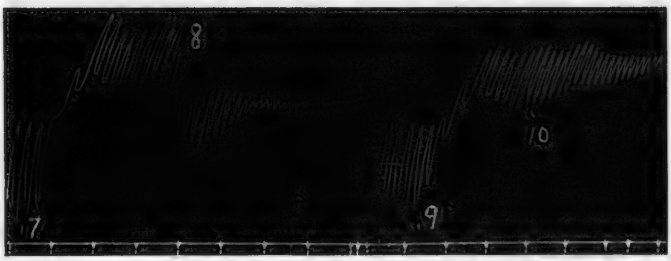

FIG. 14.

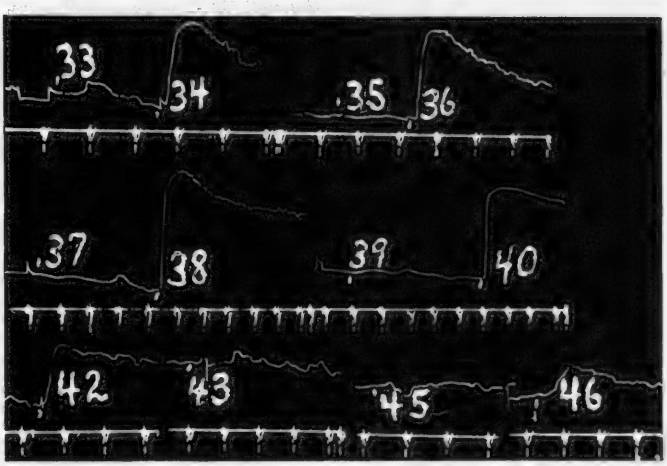

FIG. 15.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

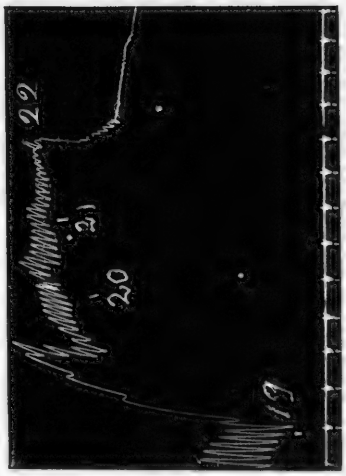

FIG. 16.

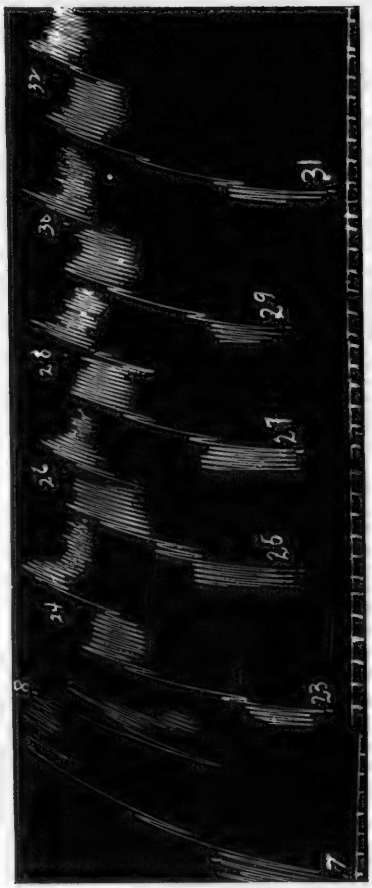

FIG. 17.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

BACTERICIDAL FLUORESCENCE EXCITED BY X-RAYS.

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INTRODUCTION.

The purpose of this article is to report an experiment which, coupled with underlying theoretical considerations, points to a mechanism by which the x-rays may be made to have a strong bactericidal effect.

It was suggested to me over a year ago that the fluorescence of dyes be studied from the point of view of determining whether there might be a relationship between this property and bactericidal action. The photodynamic effect of various dyes had previously been investigated, especially by Jodlbauer and von Tappeiner.[1] Dilute solutions of the dyes containing suspensions of various organisms were subjected to strong diffuse daylight and the results compared with controls kept in the dark. In general these experiments have given contradictory and unsatisfactory results and the influence of fluorescence as a factor was open to doubt.

Various forms of radiation have been and are being studied from the point of view of abiotic effect. Considering the spectrum as a whole from the very short or x-rays up through the ultra-violet and visible to the long infra-red and heat waves there are only two regions in which the rays have any considerable abiotic effect; namely, in the ultra-violet and infra-red. Most work has shown that the other spectral regions have very little bactericidal effect. Of these two regions the infra-red is probably not of practical value for our purposes, heat being harmful to all protoplasm. Of all radiation the ultra-violet is by far the most bactericidal and the least penetrative. Unfortunately, to be of practical value abiotic radiation must be

[1] Jodlbauer, A., and von Tappeiner, H., *Münch. med. Woch.*, 1904, li, 1096. Mettler, E., *Arch. Hyg.*, 1905, liii, 79.

capable of penetrating tissue. To alter the penetrative qualities of radiation is hopeless on the face of it.

From the point of view of utilization of radiant energy as a therapeutic bactericidal agent the problem therefore reduces itself to seeking to secure a transformation of penetrative energy into some form of energy having abiotic properties. Heretofore the attack has been along chemical lines. Transformation of energy is essentially a physical phenomenon and there is already at hand a very definite mechanism by which a transformation of this character may be produced; namely fluorescence. In order that this mechanism may be here applied, obviously two conditions must hold. In the first place, the fluorescent light, the light emitted by the substance when excited, should of itself have abiotic properties. In the second place, the exciting light, the light causing the fluorescence, must have considerable penetrative property. If these conditions hold, then abiotic light can be produced at any point to which the substance can be brought and the imposition of further physiological conditions would give a practical solution of the problem.

HISTORICAL.

The abiotic properties of radiant energy are best shown by ultra-violet light. The known ultra-violet extends from about 600 to 4000 Ångström units (0.06μ to 0.4μ). Its abiotic properties have been given considerable attention, perhaps most precisely by Victor Henri and his associates.[2] He investigated the effect on microorganisms from about 3600 down to about 2100 Ångström units, using a few filter screens to obtain an idea as to the relative effect of different portions of the region. He concluded that the abiotic effect first became appreciable somewhere between 2800 and 3000 Ångström units and increased progressively with decrease in wave-length. Lyman[3] has shown that the effect increases markedly below 2000, being almost instantaneous somewhere below 1750.

We have repeated the experiments of Henri using a more precise method and have come to the same general conclusions, placing the upper limit at about 2800 Ångström units and finding an approxi-

[2] Cernovodeanu, P., and Henri, V., *Compt. rend. Acad.*, 1910, cl, 52, 549. Henri and Henri, *ibid.*, 1912, clv, 315.

[3] Lyman, T., Spectroscopy of the extreme ultraviolet, London and New York, 1914, 103.

mately constant effect from there to 2100. In this region the typhoid bacillus is about one two-hundredth as sensitive as the photographic plate. Below 2000 Ångström units all materials become rapidly increasingly opaque. 2 cm. of water transmits at 2000, 86 per cent; at 1930, 75 per cent; at 1860, 30 per cent; and at 1729 Ångström units, 0.5 mm. of water is opaque.[4] It is therefore probably safe to set the figures 1800 to 2800 as defining the useful limits of the abiotic spectrum.

While the employment of red light as a penetrating radiation offers certain ulterior advantages, physical considerations discourage the prospect of producing ultra-violet fluorescence by this means. Fluorescence is almost invariably of longer wave-length than its excitant. A good example of this is the dye fluorescein. Dilute solutions of this fluoresce a bright green in daylight but by lamplight lose the property, lamplight containing plenty of green rays but no shorter blue waves. This same general law of fluorescence renders it probable that the x-ray should produce ultra-violet fluorescence, and in fact oftener than it does visible fluorescence. The latter type of x-ray fluorescence has long been known; the discovery of x-rays was due to this property.

The fluorescence problem thus resolves itself into the discovery or manufacture of substances which shall have desirable biological properties and at the same time emit light of wave-length less than 2800 Ångström units when subjected to x-radiation. The problem is therefore one which is rational and which at the same time offers far reaching possibilities.

It is well at this point to give an outline of the work as to the effect of x-rays alone on microorganisms. The literature on this subject seems to have stopped in 1906 with the publication by Russ[5] of a long article which also summarizes the results of previous workers. They all, with one exception, had obtained negative results, even with very long exposures to the x-rays. A great many pathogenic organisms were tried and in the presence of various culture media. Rieder[6] in 1898, using an apparatus incompletely described, was able to almost sterilize agar and gelatin plates of cholera, diphtheria, typhoid, and colon organ-

[4] Lyman,[3] p. 60.

[5] Russ, V. K., *Arch. Hyg.*, 1906, lvi, 341.

[6] Rieder, H., *Münch. med. Woch.*, 1898, xlv, 101, 773.

isms, with exposure for about 1 hour. Bouillon cultures were not so clearly affected. These results are in disagreement with those of everyone else including ourselves. A study of Rieder's article, however, does not reveal any probable cause for the discrepancy. Russ performed four types of experiments. He exposed the media to determine whether exposure rendered them unfit for culture purposes. He exposed organisms under the objective of the microscope in order to observe the effect upon their motility and clumping. He exposed the organisms in the various culture media and he inoculated exposed organisms into animals. All his experiments were entirely negative except for increased motility under the microscope in some cases. His exposures were from ½ to 2 hours, using a fairly powerful apparatus and soft, medium, and hard tubes. Our experiments incidentally check the work of Russ.

It was shown in 1905 by Schuhknecht[7] that the fluorescence in fluorite excited by x-rays extends from 2310 to 3900 Ångström units with a maximum at 2840. This mineral, therefore, should be suitable to use in experiments designed to demonstrate the essential correctness of the above considerations.

We have therefore undertaken the experiments reported in the following pages. The results embody an answer to the following questions. (1) Have the x-rays any bactericidal value and if so under what conditions is it demonstrable? (2) Can the x-rays be shown to have an increased bactericidal value when the bacteria are exposed to them in contact with a substance (fluorite) of such chemical and physical constitution that the x-rays excite fluorescence in it? (3) The second question being answered in the affirmative, is the increased activity due to chemical products contributed to the medium in which the bacteria are suspended, or is it due to physical agencies, presumably rays of light in the ultra-violet region of the spectrum?

The general conditions governing the experiments were as follows. The x-ray apparatus[8] consists of a coarse focus Coolidge tube operated by a Snook interrupterless transformer. The tube was operated on a 4 milliampere current backed up by a voltage equivalent to a 9 inch spark-gap. A 1 mm. aluminum filter plus black paper was used and the objects were placed at a distance of 7 inches from the anticathode. The doses were in multiples of 5 minutes, a 5 minute pause occurring between each 5 minute exposure. Fresh 18 hour agar

[7] Schuhknecht, P., *Ann. Physik.*, 1905, xvii, 717.

[8] The x-ray apparatus was kindly placed at my disposal by Dr. David R. Bowen of the Pennsylvania Hospital.

slant cultures of *Bacillus typhosus* were used, a suspension of the organisms in some medium, usually distilled water, being made. One of these suspensions stays nearly constant in count throughout the day. Each figure in the tables gives the average bacterial count per square centimeter for an agar plate made from the suspension in question.

In earlier experiments with small capillary tubes of glass as containers, certain specimens of glass gave much lower bacterial counts than the average. As glass of certain kinds is known to fluoresce under the x-rays, in order to avoid this effect paraffin was chosen as a sufficiently inert substance from which to construct containers. In order to demonstrate its inertness holes of two sizes were made, one $\frac{1}{2}$ inch in diameter and the other $\frac{1}{10}$ inch in diameter. These two have a considerably different ratio of wall to volume and figures obtained with them should therefore serve to demonstrate any influence of the wall. Experiments showed no difference for exposures in the two sizes of holes (Tables I and II). This at the same time serves to eliminate the size of the hole as a factor in determining the outcome of the experiment. In order to prevent evaporation from the holes they were sealed with thin sheets of paraffin.

Action of X-Rays Alone.

Table I gives the results of the exposure of water and normal salt solution suspensions of the bacteria to the x-rays.

TABLE I.

25 Minute Exposures of Salt Solution and Water Suspensions of Bacillus typhosus.

Medium.	Exposed.	Controls.	
		Before.	After (2 hrs.).
Salt solution, small hole.....	40, 43	180, 200	
Water, large hole.....	17, 12, 17, 17	55, 54, 60	60, 70, 70
" small "	15, 18, 17	61, 70	74
" " "	39, 38, 41	140, 135	130
" " "	44, 43	140, 145	

The figures are bacterial counts of a suspension of *B. typhosus* in water and normal salt solution exposed to the x-rays, the containers being $\frac{1}{2}$ and $\frac{1}{10}$ inch holes in paraffin blocks.

In my hands there has been uniformly a considerable reduction in the bacterial count of these suspensions. As the typhoid organism was supposed to be unaffected by the x-rays, experiments were made to determine the cause of this discrepancy. Water had never been used before as a medium. Usually the observations were made by exposing agar or gelatin plates. Even Rieder did not demonstrate an effect with bouillon as a medium. An agar plate does not make a good experiment. Presumably each individual organism after fixation in the media has already started to multiply before the necessary procedures incident to exposure are completed. As a result an agent which will kill 30 per cent of the organisms will presumably still leave in each potential colony at least one live organism and the full number of colonies will be produced. At any rate the plate experiments were tried using 25 minute exposures and protecting half the plate with $\frac{1}{2}$ inch of lead. The experiment was entirely negative whether the plate was incubated 1 hour before radiating or exposed immediately; that is, in about 15 minutes.

Table II shows that bouillon may have a protecting effect.

TABLE II.

25 Minute Exposures in Water and Bouillon of Bacillus typhosus.

Medium.	Exposed.	Controls.	
		Before.	After (2 hrs.).
Bouillon culture 4½ hrs. old.....	900, 1,150, 1,300	750, 700	1,500, 1,700, 2,000
" suspension, large hole.....	50, 45, 45, 47	80, 76, 80	85, 87, 92
" " small "	36, 25, 36	75, 60	70, 82
Water, large hole.....	17, 12, 17, 17	55, 54, 60	60, 70, 70
" small "	15, 18, 17	61, 70	74

The figures are bacterial counts of typhoid bacilli grown in bouillon or suspended in bouillon and in water, exposure being made to the x-rays in containers consisting of $\frac{1}{2}$ and $\frac{1}{10}$ inch holes in paraffin blocks.

A 4 hour bouillon culture exposed 25 minutes merely had its growth during the time of exposure partially inhibited. On the contrary, a bouillon suspension made just before exposure was reduced almost one-half in count. Water suspensions under similar conditions had their bacterial counts decreased to from one-third to one-fourth.

While these experiments are not conclusive as fixing the exact action of the x-rays alone, they nevertheless demonstrate the relative constancy of an effect much less potent than that presently to be described.

It should be noted in experiments of this sort that if the number of organisms dying in successive short intervals of time during which a destructive agency is acting be plotted, the points constitute a locus which tends to have the form of a probability curve, a curve having somewhat the shape of the profile of a wide flanged bell. This is the ordinary probability relationship familiar to everyone. The organisms die rapidly during part of the time causing a heaping up of the number of deaths in a short interval. In this interval of high mortality the total number killed increases very rapidly. This period is preceded and followed by intervals in which the additions to the figures for the total dead are less rapid. If the figures giving the total dead up to any time be plotted they form a locus having a different form from the above locus for rate of death. This new locus approximates a curve whose slope (tangent of the gradient angle) at any point is given by the height of a corresponding point on the probability curve, in other words by the death rate.[9] It is a curve, therefore, which starts with a low slope, gradually increasing to a maximum of steepness at the point of greatest mortality, then rising less and less rapidly to approach asymptotically a horizontal repre-

[9] The probability curve has a form given by the equation $y=e^{-x^2}$. For a mathematical discussion of the subject see the article on "Probability" in the Encyclopædia Britannica, 9th edition. In this article Fig. 1 outlines the shape of the curve. The area of each quadrilateral would represent the number of deaths occurring in an interval of time represented by the length of its short side. The total number of deaths caused by a dose acting up to any instant of time would be represented by the sum of all the preceding quadrilaterals; that is, by the area under the curve up to that point. The values of the second half of this integral from the mid-point of the curve on, are given in the table under paragraph 9. Plotted, they give the second half of the total mortality curve, the first half of the curve being the symmetrical extension backwards of this locus. It is on a curve of this form that the figures in my tables tend to lie if they are plotted against the length of dose. The Encyclopædia Britannica, 11th edition, under "Probability" gives in Fig. 10 the form of the probability curve and in paragraph 99 the table of values of its integral.

senting the total number of organisms subject to death. The total number dead increases slowly at first becoming rapidly larger as the point of 50 per cent sterilization is approached. From here on the number of dead increases less and less rapidly until finally all are dead.

Slight variations in the conditions of the experiment may shift this period of rapid sterilization a little one way or the other, thus producing excessive variations in the results of experiments ending within the period. Conversely large doses will require considerable increments to produce additional deaths. The increase in energy required to kill the last 10 per cent is greater than that required to kill the middle 50 per cent.

Influence of Fluorite.

The first experiments with fluorite were made with the native powder. They proved unsatisfactory on account of the controls, the powder alone killing a large percentage of the organisms. This was probably an agglutinating effect produced by shaking with the very fine microscopic crystals. Larger chipped crystals were tried but floating crystals here disturbed the uniformity of size of the drop on the plating loop. A large greenish crystal of fluorite weighing 122 gm. was then obtained from the collection of the Drexel Institute and into this a hole 1 cm. deep was drilled with a No. 42 drill (about $\frac{1}{10}$ inch). This hole when thoroughly washed out has smooth glass-like walls and organisms suspended in water placed in it for 2 hours were uninfluenced as shown by Table III.

TABLE III.

Influence of Fluorite Container Alone.

Control before	340, 320, 330
Fluorite hole, 2 hrs.	360, 340, 320
Paraffin " 2 "	320, 330, 360
Control after	360, 310, 340

Water suspensions of *B. typhosus* placed in $\frac{1}{10}$ inch holes in fluorite and paraffin and left there for 2 hours. The suspensions were then syringed out and plated.

Some difficulty was experienced in obtaining uniform loops of material for plating under the varying conditions. When plating from ordinary test-tubes the surface of the liquid is sufficiently large so that its surface tension does not influence irregularly that of the loop. With small holes or small drops the varying surface tension influences considerably the size of the drop on the loop. By placing equal quantities of material on cover-slips a fairly uniform loopful could be obtained. Suspensions placed in small holes in fluorite or paraffin were syringed out well and placed on cover-slips. The angle and velocity of the loop in leaving the drop on the cover-slip were kept about the same and the size of the drop was observed as satisfactory before plating. Control experiments were made with No. 42 holes in paraffin blocks. Table IV gives the results of exposures with the Drexel crystal.

TABLE IV.

Bactericidal Effect of Fluorite Fluorescence.

Exposure.	Fluorite.	Paraffin.	Control, not exposed.
min.			
15	2, 1, 2	80, 75, 75, 75	105, 110, 115, 111, 117
20	2, 3, 4,	70, 100, 100	160, 170
20	8, 7	140, 140	170, 170, 190

Exposures to the x-rays of water suspensions of *B. typhosus* in $\frac{1}{16}$ inch holes in fluorite and in paraffin.

One of the 20 minute exposures giving a 95 per cent mortality (7, 8 colonies per sq. cm.) is relatively the weakest bactericidal effect obtained with this crystal in any of the experiments. The corresponding controls in paraffin showed a mortality of from 22 to 45 per cent, depending upon the length of exposure and upon ordinary experimental variations. As indicated above the 95 to $98\frac{1}{2}$ per cent mortality found here means relatively more than the 50 per cent mortality of the x-rayed controls.

Nature of the Effect.

In order to limit the possibility of a chemical effect induced by the x-rays the procedure was adopted of placing the organisms in small

quartz capillaries and sealing with a tiny bit of paraffin at each end. This operation is entirely without effect of itself. The quartz is transparent to ultra-violet light and the arrangement limits the possible interpretations of the experiment.

The results of these exposures are given in Table V. They paralleled those made without the quartz protection. The difference between the quartz and paraffin exposures may be accidental or it may be due to some effect of the quartz itself. It is not impossible that the quartz may be slightly fluorescent under the x-rays.

TABLE V.

The Bactericidal Effect of Fluorite Fluorescence Is Not Chemical.

Exposures.				Controls.		
Exposure.	Fluorite and quartz.	Quartz.	Paraffin.	Stock.	Fluorite 2 hrs.	Quartz 2 hrs.
min.						
15	15, 14	60, 45, 40	54, 52, 48	140, 145, 130		130, 140, 130, 160
25	0, 0	40, 34	40, 43	180, 200	170, 180	
25	1, 1, 1	15, 19, 16, 17, 21, 19, 16, 18	39, 38, 41, 61	140, 135	120, 240	120, 160

Exposures to x-rays of water suspensions of *B. typhosus* enclosed in quartz tubes and placed in a hole in the fluorite crystal.

Table VI gives the results of 15 minute exposures of other crystals, with protection.

These crystals are all average specimens as seen in mineralogy collections, not being very perfect in form. They included clear white, green, and purple specimens. All were about equally active except a rather deeply colored light green crystal from Deming, New Mexico. Their visible fluorescence under the x-rays varied, being green, blue, and violet. Their weights ranged from 14 to 122 gm.

Table VII gives the results of an exposure of certain of these crystals, the suspensions being placed in the holes without quartz protection.

TABLE VI.

Variations in Crystals, Quartz Protection.

Origin.	Exposures.								Controls.	
	Fluorite crystals.						Quartz.	Paraffin.	Stock.	Quartz.
	Drexel.	Wear, England.	Deming, N. M.	Freiberg, Saxony.	Freiberg, Saxony.	Macomb, N. Y.				
Weight in gm.	122	97	37	14	52	46				
Color.	Green.	Bluish.	Green.	White.	Purple.	Slightly green.				
Fluorescence.	Green.	Blue.	Green.	Violet.	Violet.	Green.				
Bacterial count.	15, 14	16, 15	28, 35	4, 5	8, 7	8	60, 45, 40	54, 52, 48	140 145 130	130, 140, 130, 160

Exposures to x-rays of water suspensions of *B. typhosus* in $\frac{1}{10}$ inch holes in various crystals, the suspensions being protected by quartz.

TABLE VII.

Variations in Crystals, without Quartz Protection.

Exposure.	Crystals.					Controls.
	Drexel.	Freiberg (white).	Macomb, N. Y.	Deming, N. M.	Paraffin.	Stock.
15 min.....	2, 1, 2	1, 1, 1	2, 2, 2	10, 10, 8	80, 85, 75, 75	105, 110, 115, 111, 117
None.....	180, 180	140, 180	150, 160, 150	140, 160		180, 160

Exposures to the x-rays of water suspensions of *B. typhosus* in holes in the various crystals, together with 2 hour controls, without exposure.

The rather high mortality in fluorite shown here is probably not significant of anything but ordinary experimental variations.

There seems to be no insurmountable obstacle to the development of substances having this property of fluorite and at the same time being biologically more appropriate. It is hoped that a new and productive field of experimental therapy has been opened up.

CONCLUSIONS.

X-ray fluorescence has been pointed out as a mechanism offering exceptional possibilities in the development of physicochemical therapy.

Experiments are given which demonstrate that under these conditions the x-rays may have a strong bactericidal effect.

The x-rays alone have a partial bactericidal action on water suspensions of typhoid bacilli.

I take pleasure in expressing my indebtedness to Dr. Paul A. Lewis for his advice in the development of these conceptions and the carrying out of the experiments.

HEMOLYSINS OF VEGETABLE ORIGIN.

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In previous communications I reported the results of an investigation on the bacterial agglutinins, precipitins (bacterial and albugineous) and hemagglutinating substances found in the sap of *Cotyledon scheideckeri*. The present paper deals with the hemolysins which I have discovered in the sap of this plant.

2 cc. of sap in a 1: 10 dilution were mixed with 0.5 cc. of a 10 per cent suspension of sheep erythrocytes in isotonic salt solution (0.85 per cent) and were left for ½ hour at 37°C. The mixture was then thoroughly shaken, whether agglutination was observed or not, and was again left at 37°C. for ½ hour. The result is given in Table I.

TABLE I.

No. of plant.	Date of collecting leaves.	Hemagglutination.	Hemolysis.*
1	Jan. 7	+	Strong.
2	" 20	−	None.
3	" 20	−	"
4	" 20	+	Strong.
5	" 7	+	"
6	" 20	+	Present.
7	" 20	−	None.
8	" 20	+	Strong.
9	Feb. 4	+	Present.
10	" 4	+	Strong.
11	" 4	+	"
12	" 4	+	Slight.

* The three degrees of hemolysis correspond to the intensity of coloration due to hemoglobin: strong, present, and slight.

The experiments show (Table I) that hemolysis is observed only in sap that contains hemagglutinins; when the *Cotyledon* sap is deprived of hemagglutinins it does not possess hemolysins. Moreover, complete

hemolysis never appears in *Cotyledon* saps, and the hemolytic power is measured by the intensity of color of the liquid covering the erythrocytes. According to von Liebermann, ricin also never gives complete hemolysis. The experiment can be performed with ordinary test-tubes, but I used centrifuge tubes as they permit a more rapid procedure, and I submitted the suspension to centrifuging after ½ hour at 37°.

It has been stated that the agglutinated erythrocytes were thoroughly shaken in *Cotyledon* sap; and the agglutinated clot again furnished a homogeneous suspension, at least macroscopically homogeneous. This restoration of the original suspension is necessary in order to obtain hemolysis, as is shown in the following experiment.

2 cc. of sap in a 1: 10 dilution were mixed with 0.5 cc. of a 10 per cent suspension of sheep erythrocytes and left at 37° for ½ hour. After ½ hour the tubes with the sap containing hemagglutinins were left at 37° for 2 hours, without shaking the agglutinated erythrocytes (Table II).

TABLE II.

No. of plant.	Date of collecting leaves.	Hemolysis.
4	Jan. 20	None.
9	Feb. 4	"
10	" 4	"
11	" 4	"
12	" 4	"

The comparison of these results with the corresponding results in Table I proves that we cannot obtain hemolysis without thoroughly shaking the agglutinated erythrocytes.

Ehrlich and Baumgarten, cited by Fraenkel, observed similar phenomena with ricin. Von Liebermann, in his study of hemolysis in ricin, also shook the agglutinated corpuscles. Fraenkel does not consider this absolutely necessary, having observed that ricin in large amounts is able to dissolve red corpuscles directly.

Ehrlich calls attention to the fact that agglutination considerably reduced the superficies of the erythrocytes accessible to the action of the surrounding fluid and thus prevents hemolysis in the sap.

I have previously shown that the bacterial agglutinins, precipitins, and hemagglutinins of *Cotyledon* can be bound by corresponding antigens. This is also the case with the hemolysins of *Cotyledon scheideckeri*.

2 cc. of sap in a 1:10 dilution were mixed with 0.5 cc. of a 10 per cent suspension of sheep corpuscles and left at 37° for ½ hour; the mixture was then centrifuged, the sap was decanted, and the inner surface of the tube and the erythrocytes were carefully washed three times with isotonic salt solution in order to remove the remaining vegetable sap. 2 cc. of isotonic salt solution were introduced into the tube, the erythrocytes were thoroughly shaken, and the mixture was left at 37° for ½ hour.

TABLE III.

No. of plant.	Date of collecting leaves.	Hemolysis.
4	Jan. 20	Strong.
	Feb. 4	"
7	Jan. 20	"
9	Feb. 4	Present.
13	" 4	Slight.
10	" 4	Strong.
11	" 4	"
12	" 4	Slight.

The results (Table III) show that hemolysins of vegetable sap can be bound with erythrocytes; after fixation the hemolysis can take place not only in the sap of plants, but also in isotonic salt solution. In the latter case the hemolytic power remains the same (Table IV); neither in the sap nor in the isotonic salt solution do we obtain complete hemolysis, though further observations still showed that hemolysins were present in the vegetable sap after the binding of a part of the hemolysins by a suspension of erythrocytes (Table VI).

TABLE IV.

No. of plant.	Date of collecting leaves.	Hemolysis.	
		In vegetable sap.	In isotonic salt solution.
14	Mar. 6	Strong.	Strong.
15	" 6	"	"
16	" 6	"	"
17	" 6	"	"

Moreover, a comparison of Tables III and V shows that the hemolytic power of the red cells which had bound the *Cotyledon* hemolysin does not increase even when they are introduced into fresh sap containing the original quantity of hemolysins.

2 cc. of sap in a 1: 10 dilution were mixed with 0.5 cc. of a 10 per cent suspension of sheep corpuscles (0.05 cc. of sediment) and left at 37° for ½ hour; the mixture was centrifuged, the sap decanted, the corpuscles and the inner surface of the tube were washed three times with isotonic salt solution; 2 cc. of fresh vegetable sap of the same plant were introduced into the tube, the erythrocytes were thoroughly shaken, and the mixture was left again at 37° for ½ hour.

TABLE V.

No. of plant.	Date of collecting leaves.	Hemolysis.
4	Jan. 20	Strong.
	Feb. 4	"
9	" 4	Present.
10	" 4	Strong.
12	" 4	Slight.

The last two experiments led to the conclusion that either a definite quantity of red cells is able to bind only a quantity of hemolysins which is insufficient for complete hemolysis, or that the red cells are so modified during the process of binding the hemolysins by other substances contained in the sap and also by the red cells themselves that they can no longer be completely hemolyzed.

I shall return to this question after describing a series of related experiments. It seemed important to determine whether the sap possessed free hemolysins after being incubated for ½ hour at 37° with the 0.5 cc. of a 10 per cent suspension of erythrocytes remaining in the sap; in other words, how much hemolysin, measured by the amount of hemolyzed erythrocytes, was contained in the sap of each plant?

2 cc. of sap in a 1: 10 dilution were mixed with 0.5 cc. of a 10 per cent suspension of sheep corpuscles, left for ½ hour at 37°, and then centrifuged; the corpuscles recovered from the sap were washed three times with isotonic salt solution, and tested for hemolysis for ½ hour at 37° in 2 cc. of salt solution; the decanted sap was again mixed with fresh erythrocytes (0.5 cc. of a 10 per cent suspension) and

left in the thermostat for ½ hour. This was repeated as long as the erythrocytes extracted hemolysin from the vegetable sap, and consequently were undergoing hemolysis in 2 cc. of isotonic salt solution (Table VI).

TABLE VI.

No. of plant.	Date of collecting leaves.	Hemolysis. 0.5 cc. of erythrocytes.								Total quantity of erythrocytes hemolyzed.
										cc.
4	Feb. 4	Strong.	Strong.	Strong.	Strong.	Strong.	Strong.	Strong.	None.	3.5
9	" 4	Present.	Present.	Slight.	None.	None.	None.	None.	"	1.5
10	" 4	Strong.	"	None.	"	"	"	"	"	1.0
11	" 4	"	Strong.	"	"	"	"	"	"	1.0
12	" 4	"	"	Slight.	"	"	"	"	"	1.5

The experiments of Table VI show that 0.5 cc. of a 10 per cent suspension of sheep corpuscles is unable to bind the total amount of hemolysin contained in the sap.

Moreover, the amount of hemolysins found in the sap of different plants is subject to the same fluctuations as that of bacterial agglutinins, precipitins, and hemagglutinins (Table VI).

Fraenkel and von Liebermann showed that the hemolytic power of ricin increased in proportion to the quantity of this phytalbumin. These authors disagree only as to the question of the limit of this power; while Fraenkel succeeded in obtaining complete hemolysis, von Liebermann never observed it.

TABLE VII.

No. of plant.	Date of collecting leaves.	Hemolysis.	
		10 per cent suspension.	5 per cent suspension.
8	Feb. 25	Strong.	Strong.
18	" 25	"	"
19	" 25	Present.	Present.
20	" 25	Strong.	Strong.
21	Mar. 6	"	"

The experiments described above prove that the presence of superfluous hemolysins in *Cotyledon* sap has no influence upon the intensity of hemolysis and never produces the total dissolution of the red cells. Table VII gives the experiments undertaken in order to observe the hemolytic processes in undissolved *Cotyledon* sap, of course possessing far greater quantities of hemolysins than the 1: 10 dilutions used for the preceding experiments.

The procedure was the same as in the experiments given in Table I, except that a 5 per cent suspension of sheep red cells was used in addition to the 10 per cent suspension. Complete hemolysis was not observed.

Like the bacterial agglutinins, precipitins, and hemagglutinins already described, the *Cotyledon* hemolysins possess a strong avidity for red cells (Table VIII) and cannot be separated from them after 1 hour either at 45° or 75°.

0.5 cc. of a 10 per cent suspension of sheep corpuscles was mixed with 2 cc. of sap in a 1: 10 dilution and left at 37° for ½ hour; the sap was decanted, the erythrocytes and the inner surface of the tube were washed three times with isotonic salt solution, then 3 cc. of isotonic salt solution were added to the corpuscles, and the liquid was shaken and centrifuged. 1 cc. of salt solution taken after centrifuging, and consequently colored from hemolysis, did not produce hemolysis with 0.5 cc. of a 5 per cent suspension of fresh erythrocytes. It was superfluous, therefore, to wash the red cells again, which had bound the hemolysin. The cells left in the tube in 2 cc. of salt solution were shaken and heated for 1 hour at 45° and 75°, then rapidly centrifuged in the heated tubes: the salt solution was decanted and its hemolytic power was tried against fresh sheep cells, as in the experiments of Table I.

TABLE VIII.

No. of plant.	Date of collecting leaves.	Hemolysis.	
		45°.	75°.
8	Feb. 25	None.	None.
18	" 25	"	"
19	" 25	"	"
20	" 25	"	"

Table IX shows that hemolysis can be obtained not only at 37° but also at room temperature after binding the hemolysins with the erythrocytes.

TABLE IX.

No. of plant.	Date of collecting leaves.	Hemolysis.
4	Jan. 20	Strong.
7	" 20	"
9	Feb. 4	Present.
10	" 4	Strong.
11	" 4	"
12	" 4	Slight.

It has already been mentioned in connection with Table VI that saps of different plants possess different quantities of hemolysins, and this is true of all other antibodies. The same method was applied to the experiments of Table X which had been used to determine

TABLE X.

No. of plant.	Date of collecting leaves.	Solution of sap.	Sap in 2 cc. of solution.	Hemolysis.	Hemagglutination.
			cc.		
4*	Feb. 4	1 : 10	0.2	Strong.	+
		1 : 50	0.04	"	+
		1 : 100	0.02	"	+
		1 : 200	0.01	"	+
		1 : 250	0.008	Slight.	+
		1 : 300	0.006	None.	+
7*	Jan. 20	1 : 10	0.2	Strong.	+
		1 : 50	0.04	"	+
		1 : 100	0.02	Present.	+
		1 : 200	0.01	Slight.	+
		1 : 250	0.008	None.	+
9*	Feb. 4	1 : 10	0.2	Present.	+
		1 : 50	0.04	Slight.	+
		1 : 100	0.02	None.	+
10	" 4	1 : 10	0.2	Strong.	+
		1 : 50	0.04	"	+
		1 : 100	0.02	Slight.	+
		1 : 200	0.01	None.	−
12	" 4	1 : 10	0.2	Slight.	+
		1 : 50	0.04	None.	+

* The limit of hemagglutination is represented by Solutions 4, 1: 700; 7, 1: 300; and 9, 1: 200.

the quantities of bacterial agglutinins, precipitins, and hemagglutinins in *Cotyledon* sap. Solutions of the sap were prepared and the final quantity of sap that no longer contained hemolysins was observed. The method was like that described in Table III.

The next problem was to determine the influence of high temperatures upon *Cotyledon* hemolysins. The sap of each plant was heated to temperatures of 90°, 100°, 120°, 134°, and 144° for 1 hour; it was then filtered, and the experiment with the heated sap was conducted in the way described in the protocol of Table III.

Table XI represents not only the influence of high temperatures upon hemolysins, but also upon hemagglutinins.

The thermostability of the hemolysins varies in different plants, like that of the other antibodies in *Cotyledon* sap.

The suppression of the hemolytic properties at a certain temperature and their reappearance at higher ones such as 134° and 144° (Nos. 18, February 25; 19, February 25 and March 6; 20, February 25 and March 6; and 15, March 6) is a phenomenon of particular interest.

A similar fact has been described by Landsteiner and von Rauchenbichler in regard to staphylococcal hemolysins which recover their activity at 100° after inactivation at 65°. Landsteiner explains this by assuming that at 65° the hemotoxin is bound with other substances contained in the filtrate of staphylococcal cultures, and at 100° the hemotoxin is again free. We may apply this explanation to the analogous phenomenon in *Cotyledon* sap.

The reappearance of the hemolytic properties at higher temperatures after apparent inactivity makes it seem possible that still higher temperatures, which for practical reasons could not be applied, would again deliver the bound hemolysins.

We must also take into consideration the fact that the sap which recovers its hemolytic power after a temporary inactivity is able to cause complete hemolysis of sheep red corpuscles, and that the quantity of the hemolysin of sap which can be bound by the erythrocytes is sufficient for a total dissolution of the latter; if, nevertheless, we do not observe complete hemolysis, we may suppose that the quantity of hemolysins in question, instead of being directly bound with the

TABLE XI.

No. of plant.	Date of collecting leaves.	Temperature.	Hemagglutination.	Hemolysis.
		°C.		
4	Feb. 4	100	+	Strong.
		110	+	"
		120	+	"
		134	+	"
		144	+	None.
7	Jan. 20	90	+	Strong.
		100*	+	None.
8	Mar. 6	90	+	Present.
		100*	+	None.
9	Feb. 4	90	+	Present.
		100*	+	None.
10	" 4	90	+	Strong.
		100*	+	None.
12	" 4	90	+	Slight.
		100*	+	None.
18	" 25	100	+	Present.
		120	+	None.
		134	+	"
		144	−	Total.
	Mar. 6	100	+	Strong.
		120	+	"
		134	+	Present.
		144	+	None.
19	Feb. 25	100	+	Slight.
		120	+	None.
		134	+	Total.
		144	+	"
	Mar. 6	100	+	Strong.
		120	+	None.
		134	−	Total.
		144	−	"
22	" 6	90	+	Strong.
		100*	+	None.
20	Feb. 25	100	+	Strong.
		120	+	None.
		134	+	"
		144	−	Total.
	Mar. 6	100	+	Strong.
		120	+	Present.
		134	+	None.
		144	−	Total.
14	" 6	90	+	Slight.
		100*	+	None.
15	" 6	100	+	Slight.
		120	+	None.
		134	−	Total.
		144	−	"

* Even at 120, 134, and 144° there was no hemolysis. In this table some data are omitted to avoid repetition.

erythrocytes, is bound with some other substance contained in *Cotyledon* sap and bound with the erythrocytes through its second receptor, in many saps temperatures of 134° and 144° destroy this unknown substance and thus set free a part of the hemolysins; these hemolysins added to those that are bound with the erythrocytes directly are now able to cause complete hemolysis. At the same time this unknown substance, or perhaps another one destroyed at similar temperatures, modifies the erythrocytes to such an extent that it is impossible to dissolve them completely even in distilled water (Table XII). On this account we must conclude that the absence of complete hemolysis in the experiments described above is due to some substance destroyed in many saps at the temperatures of 134° and 144°, thus preventing the appearance of complete hemolysis either by binding a part of the hemolysins needed for complete hemolysis, or by a reversible alteration of the erythrocytes so as to make them inaccessible to the action of any other hemolytic agents, and of the part of hemolysins directly bound with the red cells, which perhaps would be sufficient by itself to hemolyze the red cells completely.

In order to test the hemolytic power of distilled water against sheep red corpuscles, I proceeded as in the experiments described in Table III; but distilled water was used for washing the tube and the erythrocytes, and the latter were shaken in distilled water. Both the experiment and the control were made with 0.5 cc. of a 5 per cent suspension of sheep corpuscles and 4.5 cc. of distilled water.

The experiments of Table XIII show the hemolytic power of *Cotyledon* sap against the red cells of the sheep, horse, goose, and frog. The procedure was the same as in the experiments of Table III.

Thus *Cotyledon* sap hemolyzes erythrocytes of different species of vertebrates.

TABLE XII.

No. of plant.	Date of collecting leaves.	Hemolysis.	
		5 cc. of 10 per cent suspension in (2 cc.) isotonic salt solution.	Distilled water.
8	Feb. 25	Strong.	Strong.
18	" 25	"	"
19	" 25	Present.	"
20	" 25	Strong.	"

TABLE XIII.

No. of plant.	Date of collecting leaves.	Hemolysis of erythrocytes of different species.			
		Sheep.	Horse.	Goose.	Frog.*
4	Feb. 4	Strong.	Slight.	Strong.	Present.
9	" 4	Present.	Present.	Present.	"
13	" 4	Slight.	None.	None.	None.
10	" 4	Strong.	"	Strong.	Present.
11	" 4	"	Present.	"	"
12	" 4	Slight.	Slight.	"	"

* The hemolysis of frog erythrocytes takes place slowly and repeated shaking is necessary. The binding of hemolysin also proceeds slowly; it is better to prolong it for 1 hour instead of ½ hour.

The next problem was to establish the relation between hemolysins on the one hand and bacterial agglutinins, precipitins, and hemagglutinins of *Cotyledon scheideckeri* on the other.

The experiments in Tables I and II show that the vegetable sap contains hemolysins only when it contains hemagglutinins, or, according to former studies, when it possesses antibodies in general. Table XIV shows that whenever the hemagglutinins are extracted from the sap the hemolysins simultaneously disappear.

2 cc. of sap were freed of hemagglutinins by fractional addition of different quantities of a 10 per cent suspension of sheep corpuscles and subsequent centrifugation and were mixed with 0.5 cc. of a 10 per cent suspension of sheep cells; the mixture was left for 2 hours at 37°; the cells were shaken every half hour.

TABLE XIV.

No. of plant.	Date of collecting leaves.	Hemolysis.	Hemagglutination.
4	Jan. 20	None.	—
7	" 20	"	—
9	Feb. 4	"	—
10	" 4	"	—
12	" 4	"	—

The identity of hemagglutinins and hemolysins is contradicted by the fact that the activity of hemagglutinins persists during the temporary inactivity of the hemolysins (Table XII). The opposite

phenomenon, that vegetable sap retains its hemolytic properties in spite of the absence of hemagglutinins, seems to confirm the difference of these two elements, but it can be explained even without rejecting the theory of the identity of the processes of agglutination and hemolysis (held by Ehrlich, Fraenkel, von Liebermann): as to the substance preventing hemolysis which disappeared at high temperatures, we may assume that the complete hemolysis we observe is due to some substance at once able to hemolyze and to agglutinate the red cells depending on various accessory conditions.

TABLE XV.

No. of plant.	Date of collecting leaves.	Horse serum necessary to extract precipitins (1:5).	Hemolysis.	Precipitation.
		cc.		
23	Feb. 4	0.6	None.	None.
9	" 4	0.3	"	"
10	" 4	0.3	"	"
11	" 4	0.4	"	"
12	" 4	0.5	"	"

TABLE XVI.

No. of plant.	Date of collecting leaves.	Bacteria necessary to extract agglutinins.	Hemolysis.	Hemagglutination.
23	Feb. 4	1.0 tube.*	None.	—
9	" 4	0.7 "	"	—
10	" 4	0.5 "	"	—
11	" 4	0.5 "	"	—
12	" 4	0.6 "	"	—

* A whole culture was washed off with 1 cc. of isotonic salt solution.

At any rate it is impossible to affirm categorically the identity of the hemolysins and the hemagglutinins of *Cotyledon scheideckeri;* we can only establish the parallelism of their presence in the sap and their simultaneous extraction by erythrocytes (Table XIV).

Likewise, the hemolysins are extracted from *Cotyledon* sap together with bacterial agglutinins (Table XVI) and precipitins (Table XV).

The quantity of horse serum or bacterial mass (*Bacillus typhi*) necessary for the extraction of all precipitins or bacterial agglutinins from 2 cc. of sap in a 1: 10

dilution was first determined. The previously ascertained quantity of serum (inactivated at 56°) or of bacteria was then introduced into 2 cc. of fresh sap in a 1: 10 dilution; after precipitation (30 minutes at room temperature) or agglutination (1 hour at 37°) the mixture was centrifuged, the liquid decanted, and its hemolytic properties were tested as in the experiments of Table III. An experiment was done to control the complete absence of precipitins or agglutinins.

The experiments in Table XVII show the influence of animal serum upon the hemolytic power of *Cotyledon* sap.

Horse serum inactivated for ½ hour at 56° was added to 2 cc. of sap in a 1: 10 dilution; the mixture was shaken and 0.5 cc. of a 10 per cent suspension of sheep cells was added. After centrifuging for ½ hour at 37° the liquid was decanted, and the tube and the cells were washed with isotonic salt solution. The experiment was continued like those in Table III.

TABLE XVII.

No. of plant.	Date of collecting leaves.	Serum added.	Precipitation.	Hemagglutination.	Hemolysis.
		cc.			
4	Feb. 4	0.1	+	−	−*
		0.01	+	+	−
		0.002	+	+	−
9	" 4	0.01	+	+	−
10	" 4	0.01	+	+	−
11	" 4	0.01	+	+	−
12	" 4	0.01	+	+	−

* When the experiment was conducted like those of Table I the results were the same.

The serum prevents hemolysis in vegetable sap; in these experiments the lack of parallelism between the hemolytic and hemagglutinating properties of the sap is also evident. The quantities of serum used for the experiment (0.01 to 0.002 cc.) deprived the sap of its hemolysins, but did not influence the activity of its hemagglutinins. (The lack of parallelism between hemolysis and hemagglutination in the experiments of Table X is also evident. The agglutination of erythrocytes often takes place in the absence of hemolysis.) Only large quantities of serum (0.1 cc.) destroy both these properties at once.

However, the serum prevents hemolysis only when it is introduced

into the sap before the addition of erythrocytes. In all the experiments the serum produced precipitation; on this account we may assume that the hemolysins pass into the precipitate (Table XVII).

In this experiment the serum was introduced after the red cells; hemolysis went on normally, because the red cells had time enough to bind the hemolysin before the addition of serum (Table XVIII).

0.5 cc. of a 10 per cent suspension of sheep cells was mixed with 1.9 cc. of sap; the mixture was shaken, immediately (20 to 40 seconds) afterwards serum was added, and the entire solution was shaken again. The mixture was left for ½ hour at 37°, and then centrifuged. The liquid was decanted, the inner surface of the tube and the red cells were washed with isotonic salt solution three times, and the experiment was continued like those of Table III.

TABLE XVIII.

No of plant.	Date of collecting leaves.	Serum added.	Hemagglutination.	Hemolysis.
		cc.		
4	Feb. 4	0.01	+	Strong.
9	" 4	0.01	+	Present.
10	" 4	0.01	+	Strong.
11	" 4	0.01	+	"
12	" 4	0.01	+	Slight.

The experiments of Table XVIII also show the short interval of time necessary for binding the hemolysins with the erythrocytes.

The experiments of Table XIX confirm the supposition that the serum deprives the *Cotyledon* sap of its hemolysins because it causes the precipitation of the hemolysins in the solution, and not because it is present in the sap.

TABLE XIX.

No. of plant.	Date of collecting leaves.	Serum added.	Hemolysis.
		cc.	
9	Feb. 4	0.01	Present.
10	" 4	0.01	Strong.
11	" 4	0.01	"
12	" 4	0.01	Slight.

These experiments were conducted like those of Table III, but in this case serum was added to the mixture of erythrocytes (bound with hemolysins) and isotonic salt solution. The total quantity of liquid in the experiment was 2.5 cc. Table XIX shows that hemolysis was always observed.

A series of experiments was undertaken to determine the reciprocal relation of the vegetable hemolysins and the immune hemolytic amboceptor.

0.5 cc. of a 5 per cent suspension of sheep corpuscles was sensitized for 1 hour at 37° by 1 cc. of rabbit hemolytic amboceptor against sheep corpuscles containing three hemolytic doses. The total quantity of liquid was 2.5 cc. The mixture of erythrocytes and hemolytic serum was centrifuged, the liquid decanted, and the sensitized erythrocytes were washed three times with isotonic salt solution, mixed with 2 cc. of sap in a 1: 10 dilution, and left for ½ hour at 37°. Hemagglutination was always observed in the saps. The liquid was again decanted, the erythrocytes were washed, and the cells shaken in 2 cc. of salt solution, and left for ½ hour at 37°.

TABLE XX.

No. of plant.	Date of collecting leaves.	Hemolysis.
8	Jan. 20	Strong.
9	Feb. 4	Present.
10	" 4	Strong.
11	" 4	"
12	" 4	Slight.

The presence of hemolysis in the absence of complement (Table XX) indicates the following: either the *Cotyledon* hemolysins are bound by a group of erythrocytes different from the receptor bound with the hemolytic amboceptor, or the same receptor can be bound by the vegetable hemolysin and the hemolytic amboceptor, yet the *Cotyledon* hemolysin possessing a greater avidity supplants the amboceptor.

The experiments of Table XXI confirm the second hypothesis; the erythrocytes being bound with the vegetable hemolysins, they become inaccessible to the hemolytic amboceptor; the hemolysins of vegetable sap adhere to the erythrocytes because of their greater avidity.

The experiments were conducted like those of Table III, but in this case 1 cc. of hemolytic serum containing 3 hemolytic doses was added after the erythro-

cytes were washed. The mixture was left for 1 hour at 37° and then centrifuged; the liquid was decanted and tested for the presence of the hemolytic amboceptor (1 cc. of liquid plus 0.5 cc. of a 5 per cent suspension of sheep corpuscles plus 1 cc. of guinea pig complement, left for 1 hour at 37°); the erythrocytes were carefully washed and tested as in the experiments of Table III.

TABLE XXI.

No. of plant.	Date of collecting leaves.	Hemolysis of erythrocytes bound with sap and afterward mixed with hemolytic amboceptor.	Presence of hemolytic amboceptor after centrifuging cells bound with hemolysin *Cotyledon*.
23	Feb. 4	Strong.	Total hemolysis.*
9	" 4	Present.	" "
10	" 4	Strong.	" "
11	" 4	"	" "
12	" 4	Slight.	" "

* The control experiment proved that the quantity of hemolytic amboceptor left free after sensitizing erythrocytes not modified by sap was insignificant; in the presence of 0.1 cc. of complement it caused weak hemolysis with abundant precipitation of erythrocytes on the bottom of the tube.

Further experiments (Tables XXII and XXIII) also confirm the hypothesis that the same receptor forms the object of binding for the vegetable hemolysin as well as for the hemolytic amboceptor, and that in consequence the two hemolysins cannot be bound with the red cells simultaneously, because the hemolysin possessing a greater avidity leaves no place for the other one.

The experiments of Table XXII prove that the complement is unable to produce complete hemolysis of red cells sensitized by the hemolytic amboceptor and afterwards modified by *Cotyledon* sap.

Experiments were conducted like those of Table XX; the sensitized red corpuscles were submitted to the action of *Cotyledon* sap, carefully washed, and thoroughly mixed with 1 cc. of guinea pig complement in a dilution of 1:10. This quantity of complement during ½ hour caused complete hemolysis of 0.5 cc. of a 5 per cent suspension of sheep corpuscles, in the presence of three hemolytic doses of amboceptor, the total quantity of liquid in the tube being 2.5 cc. The tube was then filled with isotonic salt solution until the quantity of liquid was 2.5 cc., and was left for ½ hour at 37°.

TABLE XXII.

No. of plant.	Date of collecting leaves.	Hemolysis.
9	Feb. 4	Present.
10	" 4	Strong.
11	" 4	"
12	" 4	Slight.

In the present case we must attribute the absence of complete hemolysis to the disappearance of the hemolytic amboceptor from the erythrocytes and not to the incapacity of the hemolytic system to dissolve completely the erythrocytes modified by the sap. This is proved by the fact that the complement is left free in the liquid (Table XXIII).

Experiments were conducted like those of Table XXII, but in this case only 1 cc. of complement was added to the sensitized red cells altered by the vegetable sap. The tube, not filled with salt solution, was shaken and left for ½ hour at 37°. After centrifugation the liquid was decanted and tested in order to discover the presence of the complement (1 cc. of liquid plus 1 cc. of hemolytic serum in three doses plus 0.5 cc. of a 5 per cent suspension of erythrocytes).

TABLE XXIII.

No. of plant.	Date of collecting leaves.	Presence of complement in guinea pig serum after elimination of sensitized erythrocytes modified by *Cotyledon*.
23	Feb. 4	Total hemolysis.
9	" 4	" "
10	" 4	" "
11	" 4	" "

The hemolysins like the bacterial agglutinins, precipitins, and hem-agglutinins, were left when the sap, in a 1: 10 dilution, was filtered in a Chamberland filter. These experiments were conducted with the sap of Plants 8, 18, 19, and 20, on February 25.

CONCLUSIONS.

1. The sap of *Cotyledon scheideckeri* possesses hemolysins for the red corpuscles of different animals.

2. The hemolysins of vegetable sap can be bound by erythrocytes and cannot be separated.

3. A definite quantity of erythrocytes is able to extract from the sap only a part of the hemolysins it contains.

4. The quantity of hemolysins in the sap of different plants is subjected to the same fluctuations as that of bacterial agglutinins, precipitins, and hemagglutinins.

5. As the hemolysins are bound by erythrocytes, the hemolysis can take place not only at 37° but also at 15–16°.

6. The thermostability of the hemolysins varies from one individual plant to another.

7. In many cases the vegetable sap loses its hemolytic properties at a certain temperature and recovers them at a higher one.

8. The vegetable sap is unable to produce complete hemolysis of erythrocytes.

9. But the sap of many plants acquires the power to dissolve red corpuscles completely after 1 hour of heating at 134° and 144°.

10. Erythrocytes modified by *Cotyledon* sap cannot be dissolved completely even by distilled water.

11. The agglutination of the erythrocytes and their hemolysis are conditioned, probably, by different substances.

12. The hemolytic amboceptor and the hemolysin of *Cotyledon scheideckeri* can be bound with the same receptor of the erythrocytes.

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STUDIES ON THE DEVELOPMENT OF TOXICITY IN INTESTINAL SECRETION.

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(Received for publication, May 25, 1917.)

The following study was conducted with the purpose of further investigating the production of toxic material in closed loops of high intestine in dogs. These experiments are a continuation of the work of Davis,[1] and of Stone, Bernheim, and Whipple.[2] In the work of Davis, intestinal secretion, collected by irrigation of the duodenojejunum, excluding pancreatic secretion, was kept under chloroform and toluene a number of days in a warm place, and studied for its toxic properties. These fluids were found to be toxic, and it was felt that the intestinal secretion was, possibly, toxic at the moment of its elaboration. In the light of subsequent work, however, the author feels that his conclusions are subject to revision. The condition under which the fluid was kept did not absolutely exclude the possibility of bacterial growth. Whipple, Stone, and Bernheim[3] studied the toxic properties of fluids collected in closed loops of intestine left *in situ*, and certain inferences were reached as to the source of the toxin in these fluids. The present article deals with a further effort to investigate these inferences, in consequence of which light has been thrown on them from a different point of view. In the work referred to, the rapidity with which toxins developed,

[1] Davis, D. M., Intestinal obstruction: Formation and absorption of toxin, *Bull. Johns Hopkins Hosp.*, 1914, xxv, 33.

[2] Stone, H. B., Bernheim, B. M., and Whipple, G. H., The experimental study of intestinal obstruction, *Ann. Surg.*, 1914, lix, 712.

[3] Whipple, Stone, and Bernheim, Intestinal obstruction. I. A study of a toxic substance produced in closed duodenal loops, *J. Exp. Med.*, 1913, xvii, 286; II. A study of a toxic substance produced by the mucosa of closed duodenal loops, 307.

the generally assumed paucity of bacteria in high intestinal loops, and certain other reasons, led to the assumption that the activity of the mucous membrane of closed loops was the essential factor in toxin production, and that bacteria probably played a minor part, if any, in this process. Our present data justify the conclusion that toxins indistinguishable, as far as the symptoms and anatomical lesions produced by them are concerned, from those found in closed loops, may be elaborated from intestinal secretion *in vitro*, and hence make questionable the theory of the essential part played by the living mucosa of closed loops.

Toxicity of Intestinal Secretion Heated to 90–95°C.

As a point of departure for this work we desired to determine whether intestinal loop secretion, obtained in such a manner that it was as nearly normal as possible, was in itself toxic. In order to do this, it was necessary not only to make an effort to exclude bacterial action, as by the addition of chloroform and toluene, but also to eliminate the action of enzymes present in the secretion. For this purpose the following experiment was devised. Under anesthesia, the intestine was divided just distal to the lower pancreatic duct, and again at a point in the high jejunum so as to isolate a loop of duodenojejunum. The upper and lower ends of this loop were brought out of the abdominal cavity, and connected with rubber tubing so that the isolated loop could be washed through and the washings collected. The loops were irrigated with distilled water. A given volume of water, usually 1 liter, was passed through the loop repeatedly so that large quantities of fluid would not have to be handled. A number of experiments were carried out with this operative technique, the loop being washed by a slow, continuous stream for periods ranging from $3\frac{1}{2}$ to 10 hours. The washings were collected over a boiling water bath, the temperature in the collecting flask being 90–95°C. This temperature may be assumed to be destructive of both enzymes and bacteria, so that fluid so collected and reheated for $\frac{1}{2}$ hour on the 2 succeeding days, to allow for the presence of spore-bearing organisms, remains unchanged, if kept with aseptic precautions. Fluid so collected was injected intravenously into small

dogs, in two experiments, and produced no symptoms whatever. In other experiments, conducted under anesthesia, injection of this fluid caused no change in blood pressure.

Toxicity of Unheated Intestinal Secretion.

It might be suspected, however, that the heating itself destroyed a toxin normally present in intestinal secretion. To eliminate this possibility, the washings were collected as described above, except that they were not heated. This fluid was immediately injected intravenously into a small dog, also without the production of any symptoms. In addition, in two other experiments, similar unheated fluids, which had been preserved for a time by the addition of chloroform and toluene, were injected after driving off these substances at a comparatively low temperature with the aid of reduced pressure, without producing symptoms. From these experiments, we concluded that the intestinal secretion, as produced in these loops is devoid of toxic properties, and that if it is heated immediately at 90–95°C. and kept sterile, the development of toxic properties is prevented.

The next step was to discover whether this non-toxic fluid would take on, outside the body, the toxic properties characteristic of closed loop contents. Unheated loop washings, collected in sterile flasks and kept as far as possible from external contamination, were incubated at 37°C. for 18 hours. The fluids at this time showed evident and profuse bacterial growth. Upon intravenous injection, in two experiments, these incubated washings caused the death, in about 6 hours, of small dogs. In both cases the symptoms and anatomical pictures were the same as those seen after the injection of closed loop contents. The small intestine was intensely injected and hemorrhagic, most markedly in the upper duodenum. In one case, the quantity used represented the collection during 40 minutes, and in the other during 80 minutes.

Toxicity of Unheated Intestinal Secretion Treated with Chloroform and Toluene.

The development of toxic properties by the loop washings might be ascribed to the influence of bacterial growth or to the action of

enzymes, probably proteolytic, derived from the intestinal mucosa. In either case, the substrate would have to be the proteins of the succus entericus, no other substance except distilled water being present. A method of differentiation by which either enzymic or bacterial action alone could be made responsible for the change is difficult to devise. Passage of loop washings through a Berkefeld filter was not attempted, since the adsorption of a large part or all of the enzymes by the material of the filter might be expected.

Fractional sterilization of the loop washings offered little, since growth of bacteria occurs between sterilizations, and in addition the temperature of sterilization approaches that at which enzymes are injured.

Finally experiments were carried out in which unheated loop washings were treated with chloroform and toluene. Portions, in one case one-half the amount collected in $3\frac{1}{2}$ hours, and in one case one-third the amount collected in 4 hours, were kept several days and then freed of the preservative substances by distillation. The second portion had in addition been incubated at 37°C. for 18 hours. It was hoped that this procedure, while preventing bacterial growth, would allow opportunity for any enzymes present to act. The fluids were injected intravenously immediately after the distillation, and no symptoms of poisoning were observed in the dogs used.

The other portions of loop washings from the experiments concerned were incubated for 18 hours at 37°C., after being freed from the preservatives by distillation at reduced pressure, and then injected. In one case in which the distillation was carried out at a temperature of about 80°C. the injection of the fluid in a test dog produced no symptoms of poisoning. In the other, a better vacuum was obtained, and the temperature of the distillation never exceeded 60°C. The injection of this fluid produced a period of shallow respirations and weak pulse during the injection, but at the end the animal was in good condition and ran about the floor before being put in the cage. Extreme prostration supervened in 4 hours and the dog died during the ensuing night. There was no diarrhea. At autopsy the intestine was pale and showed nothing abnormal except the presence of a rather large amount of gas. The content of the small intestine was a bile-stained fluid. The mucosa showed no

hemorrhage or congestion. This picture is entirely unlike that caused by the injection of closed loop fluid, and has never been observed by either of the authors in long series of such injections.

In both these last experiments, the fluids, after removal of the preservatives and incubation for 18 hours, showed growth of *Bacillus subtilis* in spite of the precautions taken. It is probable that the spores of this organism survived the treatment with chloroform and toluene.

From these experiments, we cannot say positively that bacteria are responsible for the development of toxicity in loop washings, but they offer no support to the conception that enzymes are the cause of such development. These experiments, together with the previous ones reported by one of us (Davis[4]), show that loop washings can become toxic outside the body, producing symptoms like those caused by closed loop fluid. In this case there can be no question of the perverted activity of the epithelial cells of the mucosa. In addition, it is clear that unless chloroform and toluene inhibit the enzymes in this intestinal fluid, their action alone does not produce the toxic properties.

SUMMARY.

1. Intestinal secretion, collected by the method described in this paper, is non-toxic when fresh.

2. This secretion, when heated immediately to 90–95°C. and kept sterile, remains non-toxic.

3. This secretion, when not heated, remains non-toxic when kept under chloroform and toluene, even if incubated at 37°C.

4. This secretion, when not heated, but collected in a sterile flask, becomes toxic upon incubating 18 hours, producing symptoms like those of closed loop fluid.

5. The secretion, when treated with chloroform and toluene, and later incubated for 18 hours, after these preservatives have been removed by distillation at 60°C., does not produce lesions typical of closed loop fluid.

[4] Davis, Natural immunity of animals against poison of intestinal obstruction, *Bull. Johns Hopkins Hosp.*, 1914, xxv, 39.

A COMPARATIVE STUDY OF SERUM AND LYMPH FERMENTS AFTER FEEDING.

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(Received for publication, June 2, 1917.)

In a preceding paper Jobling and his associates[1] have discussed the ferment changes that are observed in the serum of dogs after feeding. During the course of that work certain abrupt alterations in the titer of the ferments were occasionally encountered, changes that seemed to us to appear more rapidly than would be anticipated from gradual absorption following the stimulation of the ferment-producing organs.

In order to study these relations more fully we have undertaken a comparison of the lymph and serum alterations following feeding, collecting the lymph from a thoracic duct fistula and taking samples of the blood at varying time intervals.

The technique used was the same as described in the previous paper[1] except that the action of the proteolytic ferments has been accelerated by incubating at from 47° to 50°C., which represents more nearly the optimum temperature than 37°C., heretofore used. The peptidase (ereptase) has been estimated by the dilution method with the digestion of a peptone solution and subsequent production of the tryptophane reaction with bromine water.

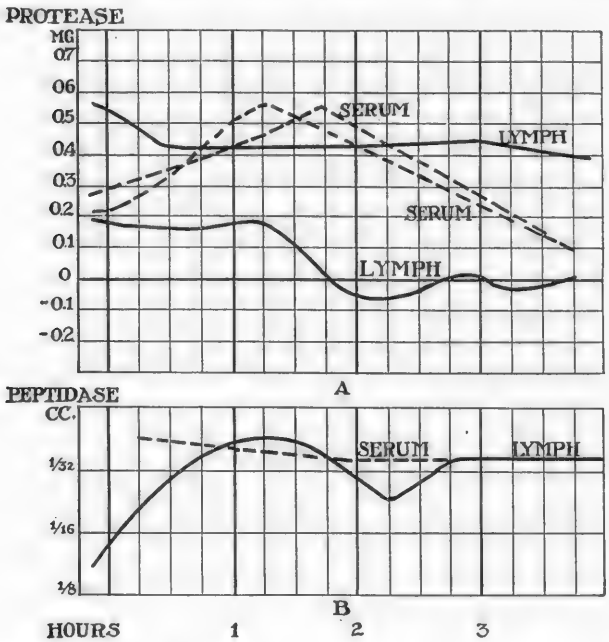
EXPERIMENTAL.

Normal dogs of from 15 to 20 kilos were operated on as a rule in the morning, a lymph fistula was established, and recovery from the operation permitted. A liter of fresh milk was then fed. The lymph was completely collected in half hour periods; the blood was taken at various time intervals.

[1] Jobling, J. W., Petersen, W., and Eggstein, A. A., *J. Exp. Med.*, 1915, xxii, 129.

Protease (*Text-Fig. 1*).—The serum protease was found to increase uniformly until a maximum was reached between the 1st and 2nd hours after feeding, following which a progressive decrease occurred until in some animals no protease action could be determined in the serum.

The lymph protease decreased progressively following the feeding, in some experiments quite rapidly, so that the negative phase of the

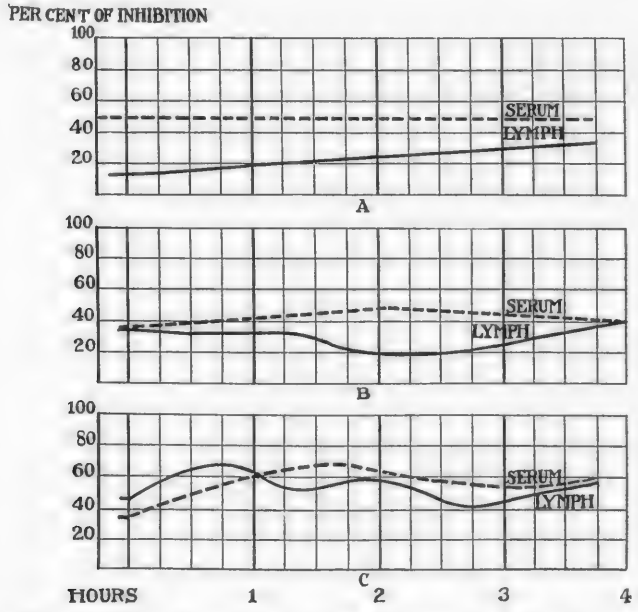


TEXT-FIG. 1. Effect of feeding on serum and lymph protease and ereptase (peptidase).

ferment action (proteosynthesis?) appeared within 2 to 3 hours after the feeding.

Peptidase.—The peptidase of the lymph is increased rapidly as a rule after the feeding, reaching a maximum at about the time that the maximum flow of lymph is observed. The peptidase of the serum, on the contrary, gave no evidence of an increase after the feeding (Text-fig. 1).

Antiferment.—In the previous experiments wide fluctuations were noted in the antiferment titer, which are readily understood when the fact that the antiferment titers of the serum and lymph are not necessarily equal is taken into consideration, so that the volume of the lymph flow entering the circulation appreciably modifies the antiferment titer of the serum.

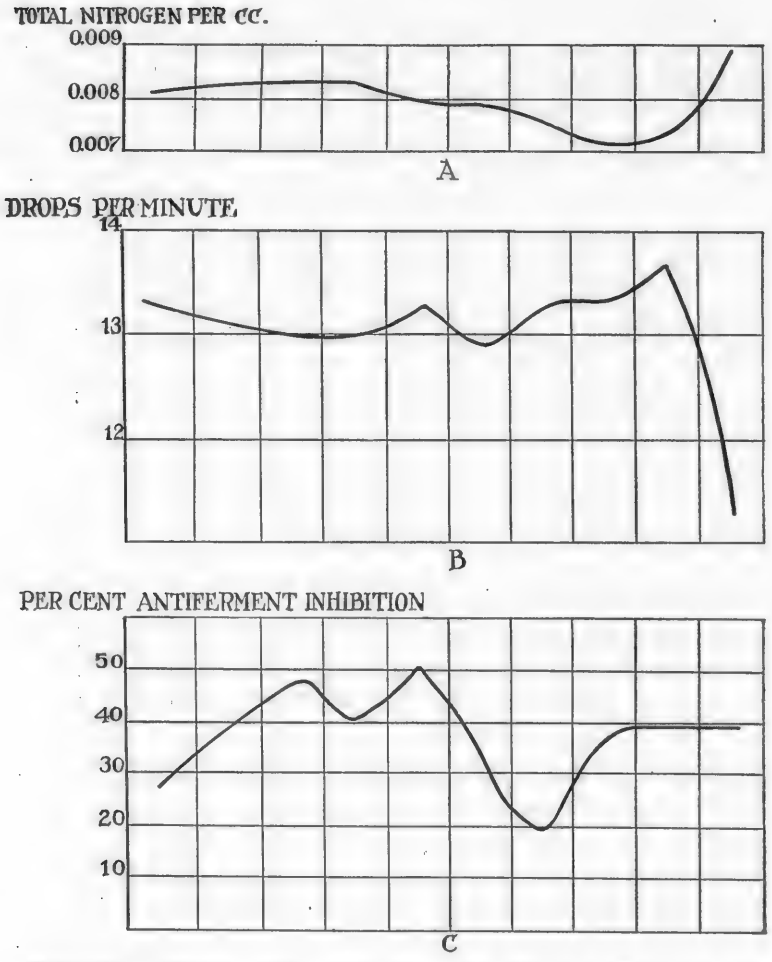


TEXT-FIG. 2. Effect of feeding on the antiferment titer of the lymph and serum.

The milk feedings used in these experiments caused a gradual increase in the antiferment titer of the lymph, but did not influence the titer of the blood serum (Text-fig. 2, A).

In the next experiment the milk fats were removed and 75 cc. of olive oil added to the milk in their place. It will be observed that in this case the antiferment titer of the lymph actually decreased, the serum showing a very slight increase (Text-fig. 2, B).

In Text-fig. 2, C is illustrated the effect of feeding 5 gm. of sodium



TEXT-FIG. 3. Relation of the antiferment to the lymph concentration and stalagmometric determination.

oleate, preceded by neutralization of the gastric acidity. In this case the antiferment both of the lymph and of the serum increased considerably.

Relation of the Antiferment to the Lymph Concentration and Stalagmometric Determinations.—Coincident with these experiments we have studied the relation of the antiferment to the concentration of the lymph as determined by the total amount of proteins contained, and also to the rate of flow through the stalagmometer. As will be observed in Text-fig. 3, the stalagmometric rate is influenced markedly by the concentration of the lymph. The antiferment on the other hand bears no relation to these two curves and its titer is obviously independent of the concentration of the proteins.

DISCUSSION AND SUMMARY.

In these experiments in which the lymph and serum ferments and antiferment have been studied separately, the changes that are found to occur are uniform and consistent. Possibly as a result of increased blood flow through the ferment-producing organs a moderate amount of protease is directly absorbed into the blood stream, but when intestinal digestion is actively under way this rapidly diminishes in extent. If any protease is absorbed during digestion from the gastrointestinal tract it is probably removed when it reaches the liver. The ereptase, or peptidase, is evidently absorbed directly from the intestinal tract and enters the circulation through the lymphatic channels.

The influence of the diet on the antiferment of the lymph is striking and accounts for the fluctuations observed in previous experiments. Following the milk meal the increase occurs gradually in the lymph in an amount that, when diluted in the blood stream, would be only nominal. When the fats of the milk were replaced by olive oil in large amounts it is surprising to find a decrease in the antiferment instead of an increase in titer, as might be expected in view of the nature of the antiferment. This result, however, is probably due to the fact that the antiferment lipoids of the serum and lymph may exist in both water and fat dispersion phases, but are active as antiferments only when in the former. If the amount of the fats of the

serum is increased, as it is after the olive oil feeding, more of the antiferment will enter the fat phase and will as a result be rendered inactive. When the feeding included the sodium oleate, the antiferment was in consequence increased in both the serum and the lymph, some of the soap being apparently absorbed directly into the blood stream. It is possible that the titer of the antiferment may be altered, therefore, by means of selective feeding.

A COMPARATIVE STUDY OF LYMPH AND SERUM FERMENTS DURING PROTEIN SHOCK REACTIONS.

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(Received for publication, June 25, 1917.)

During the course of investigations concerning the mechanism of recovery following the so called "protein shock therapy" we have been interested in determining the comparative changes that occur in the lymph and blood serum and the possible manner in which these changes are brought about.

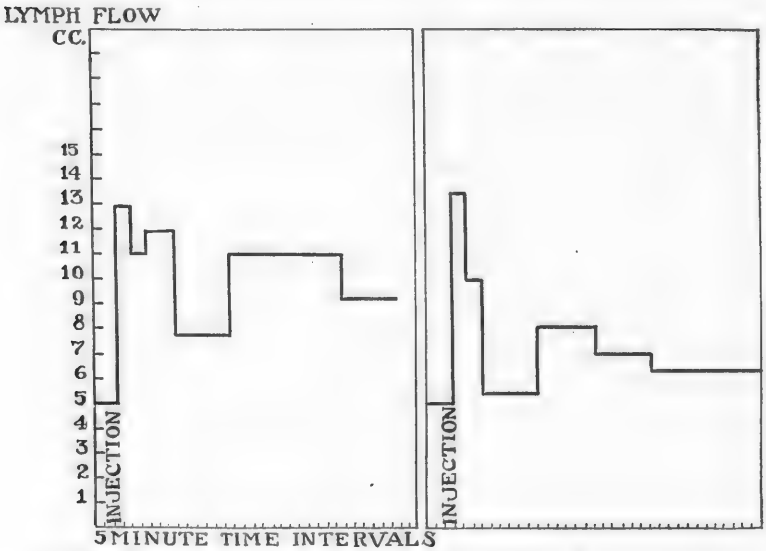
It is well known that certain substances, which Heidenhain (1) classified as lymphagogues of the first class, among them peptone, egg albumin, tissue extracts, etc., cause a marked increase in the lymph flow, supposed to be derived largely from the liver. This increased flow may continue a considerable time following such injection. Teague and McWilliams (2) have recently advanced the interesting explanation that this phenomenon is responsible for the therapeutic effect of the protein shock in that the antibodies of the blood are forced into the lymph spaces and there destroy the invading organisms. There is no doubt that the bacterial injections used in these experiments (*Bacillus coli*) do bring about a great augmentation of the lymph flow, as will be observed from the charts, and the explanation of Teague and McWilliams may well account in a large measure for the therapeutic effects.

EXPERIMENTAL.

The technique used has been identical with that described in the previous papers. Dogs of from 20 to 40 kilos were anesthetized, a thoracic duct fistula was established, and after recovery from the operation killed colon bacilli were injected intravenously, the amount used for the shock varying from two to three slants of a 24 hour

agar culture. When necessary small doses of morphine were given, although we have endeavored to avoid such measures. If the animals are injected too soon after the operation and before complete recovery has been made from the anesthetic, considerable resistance to the shock may be manifest and the temperature reaction delayed for several hours.

Lymph Volume.—The increase in the rate of flow of the lymph follows immediately upon the injection (Text-fig. 1) and in severe



TEXT-FIG. 1. Volume of lymph flow following *Bacillus coli* vaccine injection.

intoxications two periods of maximum flow seem to occur, the first immediately after the injection and persisting for from 20 to 30 minutes, the second after approximately 1 hour, the latter increase being continued over a longer period of time. When the intoxication is not so great the two phase curve does not occur, the increase being less in extent but persisting for a longer period of time.

Concentration of the Lymph and Serum and the Relation to the Stalagmometric Determination.—The concentration of the lymph following the injection is considerably increased, as determined by the

Kjeldahl method for total protein nitrogen, although the concentration of the non-protein nitrogen may decrease, as shown in Table I.

TABLE I.

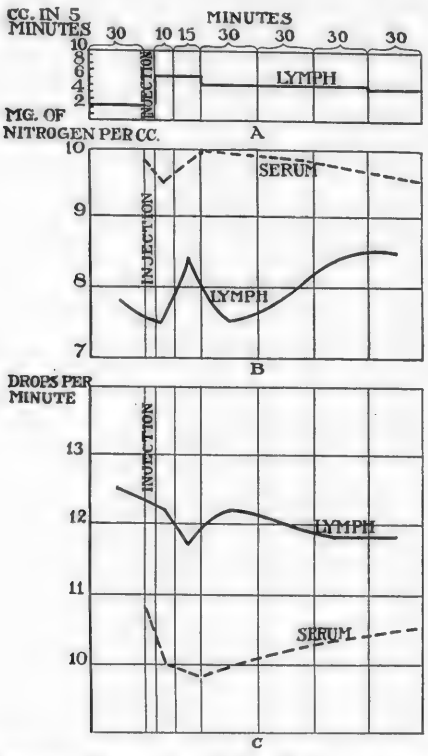
Time of sample.	Blood.		Lymph.	
	Total nitrogen per cc.	Non-protein nitrogen per cc.	Total nitrogen per cc.	Non-protein nitrogen per cc.
a.m.	*mg.*	*mg.*	*mg.*	*mg.*
11.30–12.00 (before injection) 12 n. 2 slants of colon bacilli injected.	9.8	0.45	7.8	0.5
p.m.				
12.05–12.15	9.5	0.50	7.5	0.45
12.15–12.30	9.7	0.45	8.4	0.45
12.30– 1.00			7.5	0.45
1.00– 1.30	9.8	0.50	7.8	0.47
1.30– 2.00			8.4	0.35
2.00– 2.30	9.5	0.50	8.5	0.35

The effect of the concentration of the lymph is observed directly in the rate of the stalagmometric flow, which is decreased when the concentration becomes greater. This does not always hold true for the blood serum, however, as shown in Text-fig. 2. A considerable diminution of the number of drops per minute was observed, without a corresponding increase in the concentration of the serum. Such a change must be due to an alteration in the dispersion of the serum colloids.

Antiferment.—In a preceding paper (3) it was shown that considerable fluctuations are to be observed in the antiferment titer of the lymph following feeding. The changes noted following protein shock make it probable that the antiferment is supplied to the blood stream wholly by way of the lymphatics, and not directly from the cells to the blood. In Text-fig. 3, A and B, are illustrated two curves of the antiferment index (0.05 cc. of serum and lymph) following intravenous bacterial injection.

In Text-fig. 3, A will be observed the prompt and marked rise in the antiferment of the lymph stream while the titer of the serum remains unaltered. Text-fig. 3, B shows a more gradual increase in the antiferment of the lymph with a coincident fall in the titer of

the blood serum. The increase in the antiferment observed in the lymph persisted in our experiments for the duration of the time under observation, usually about 5 hours. The increase probably would continue if the animal were permitted to live for a longer period

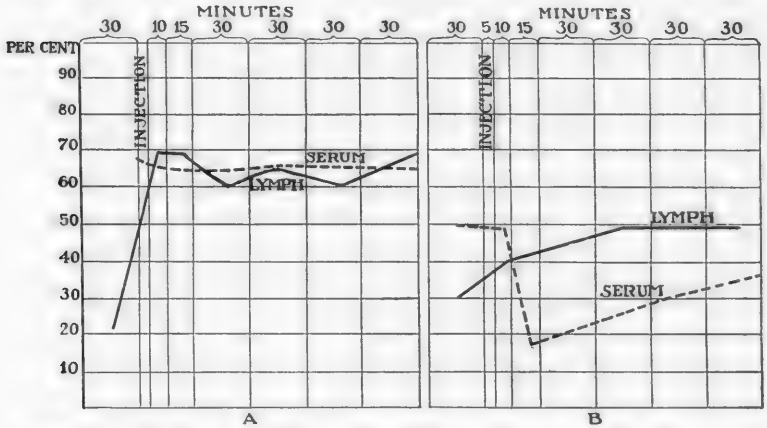


TEXT-FIG. 2. Relation of serum and lymph concentration and stalagmometric determinations. A, volume of lymph; B, concentration expressed as mg. of nitrogen per cc.; C, stalagmometric determination.

of time. No relation to the stalagmometric determination or to the protein concentration of the fluids was observed.

Protease.—The effect of the bacterial shock on the protease content of the lymph and serum is marked in extent. Three types of reaction may be distinguished: (*a*) the fluctuations in titer may

occur simultaneously, (*b*) those of the serum may precede those of the lymph, and finally, (*c*) there may be no relation of the one to the other, as illustrated in Text-fig. 4, C, in which the protease of the lymph increased and that of the serum diminished. Until more information concerning the origin and the distribution of these ferments in the vascular channels is available, it seems useless to endeavor to interpret these findings.

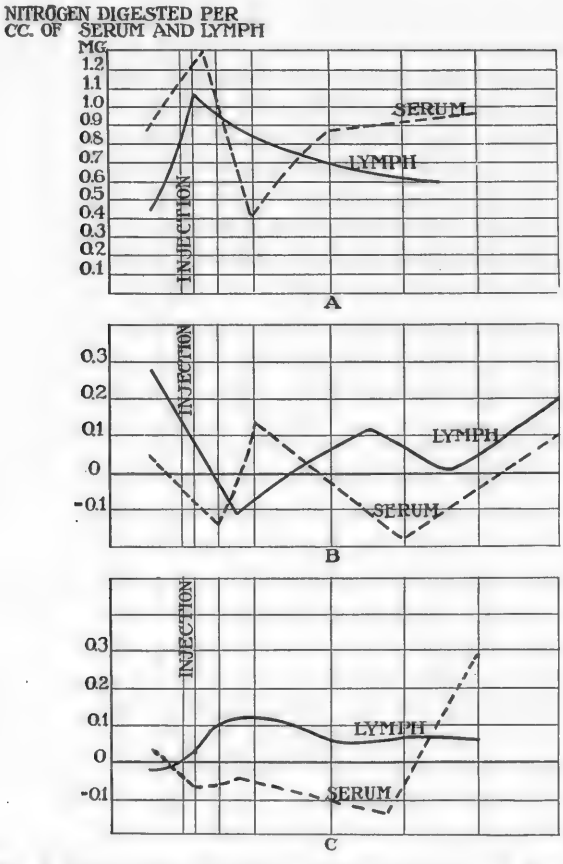


TEXT-FIG. 3. Antiferment of the lymph and serum following vaccine injections. A and B represent two different types of reaction.

Peptidase.—The fluctuations of the peptidase, or ereptase titer, do not parallel those of the protease; indeed the curves may be quite dissimilar. As a rule the increase makes its appearance later than that of the protease and is less extensive. When alterations in titer do occur they appear almost simultaneously in both the lymph and serum, although in a few experiments the ferment was first to be observed in the serum. It is at any rate apparent that the entrance into the blood stream can be direct and does not need to take place *via* the lymph channels, although under normal conditions, *i.e.*, feeding, this seems to be one portal of entry.

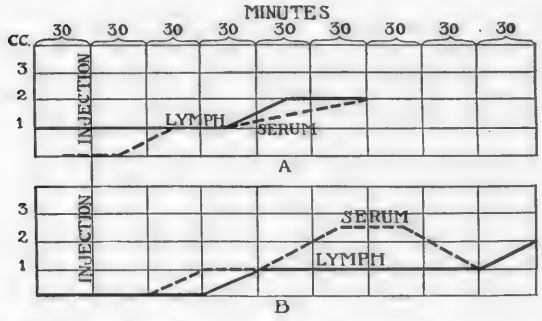
Lipase.—While the increase in this ferment occurs in both lymph and blood following shock, it seems to make its appearance first in the serum (Text-fig. 5). In Text-fig. 5, A it will be observed that

while the lymph titer was greater than that of the blood at first, the increase was first apparent in the serum, although the final titer



TEXT-FIG. 4. Protease titer following vaccine injections. A, B, and C represent three different types of reaction.

was equal in both lymph and serum. In Text-fig. 5, B is illustrated an experiment in which the increase occurred primarily and to the greatest extent in the serum.



TEXT-FIG. 5. Lipase titer following vaccine injections expressed in cc. of $\frac{N}{50}$ sodium hydroxide. A and B represent two different types of lipase reaction.

Diastase.—The diastatic activity of both the serum and lymph remained practically equal and without change following the bacterial injections in all the experiments.

DISCUSSION AND SUMMARY.

The relation of two phenomena involved in the mechanism of recovery following protein shock therapy is shown in these experiments to be due to changes that concern the lymph rather than the serum. The first of these, the increase in the rate of flow of the lymph, has been suggested by Teague and McWilliams as a possible factor in recovery from infection when due to bacteria proliferating in the lymph spaces and inaccessible to the antibodies of the serum (typhoid). By means of the protein shock, antibody-rich fluids (serum) are forced into the lymph channels. That the antibodies of the serum are augmented following the shock has been demonstrated by Culver (4). With this possibility in mind it is to be expected that bacterial infection not confined to the lymph spaces will not be influenced by shock therapy to the same extent. How far this holds true we are unable to state, although von Decastello (5) has called attention to the fact that while he was able to cause rapid lysis or a crisis in typhoid patients following the shock reaction, the injection of a similar amount of vaccine in typhus fever was without effect. The second factor, the great increase in the antiferment, is clearly due to the amount of the antiferment entering the general

circulation through the lymph stream. This accounts for the marked fluctuations observed in the titer of the serum antiferment in patients following protein shock. If, for instance, the original titer of the lymph is less than that of the blood, the first flushing of the lymphatic current into the blood channel will tend to lower the titer of the serum, but with the increased amount in the lymph after the shock the titer of the serum will also increase.

Bacteria proliferate best where the antiferment is absent, as Wright (6) has noted in his studies on war wounds, a fact that is also commonly applied in bacteriological technique when we employ ascitic fluids, containing relatively little antiferment, preferably to serum, in culture media.

The increased lymph flow that follows the shock reaction would have value then, not only in forcing specific antibodies into the lymph channels, but in increasing the antiferment there as well, which would aid in checking the growth of bacteria.

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STUDIES IN GLOMERULONEPHRITIS.

III. An Attempt to Produce Glomerulonephritis by Repeated Injections of Bacteria.

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Plate 54.

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The present paper records an effort to reproduce glomerular nephritis in animals by inoculations of bacteria, especially by repeated inoculations such as might be expected to evoke phenomena of immunity, allergy, or sensitization. The indication for the attempt is found in the hypotheses put forth by several writers in explanation of diffuse glomerulonephritis, especially that occurring as a late sequel or complication of scarlet fever.

Volhard and Fahr state that it is characteristic of diffuse nephritis that it appears at a time when immune processes are active and speak of a "diffuse toxic action." Ophüls has suggested that the glomerular endothelium acquires strong bacteriolytic properties which cause an "explosive" reaction when the bacteria are again introduced into the tissue, lesions being caused by disruption products of the bacteria. The most elaborate development of the theory is that of von Pirquet and Escherich and Schick, who apply it also to the other late complications of scarlet fever. It may be summarized as follows: At the beginning of the disease, during the acute symptoms, the virus circulates in the body, sensitizing the cells in the same way as do all the foreign proteins. The immune substances (ergins) begin to appear at the end of the 2nd week. At this time a reinvasion of the body from various foci occurs, a specific reaction or combination between antigen and antibody takes place, and this, after the latent period common to allergic reactions in general, leads to active signs and symptoms of the scarlatinal sequelæ. The latter actually occur in the 3rd and 4th weeks, between the 19th and 22nd days. It is also part of the theory that the smaller the amount of protein acting in the preliminary sensitization, the sharper will be the later allergic reaction.

Von Pirquet and Escherich and Schick state that in the case of scarlet fever experimental proof of the allergic hypothesis must await the discovery of the

specific virus of the disease, but Löhlein asserts that streptococci are either demonstrable or probably associated in all cases of diffuse nephritis, and it is commonly accepted (Holt and Howland) that many of the complications of scarlet fever, including nephritis, are probably due to secondary infection with streptococci.

It therefore seemed logical to attack this problem, in a way somewhat like that in which arthritis was previously studied, by investigating the effect upon the kidney of repeated inoculations of streptococci. In view of the fact that streptococci cause the production of only small amounts of recognizable antibodies in the serum, it was conceived that a more decisive test of the part played by humoral bacteriolysis[1] at least, would be furnished if bacteria (*Bacillus coli communior*) capable of evoking large amounts of serum antibodies were used. As a still further test, the effect of repeated large doses of these bacteria in a highly immunized and therefore resistant animal was investigated. Finally, several strains of *Staphylococcus aureus* were used for repeated inoculation.

EXPERIMENTAL.

With the exception of *Bacillus coli communior*, all the bacteria used in the following experiments were isolated from the throats of patients in the 1st week of scarlet fever. The first inoculation was usually from the second or third generation. In general, the organism found in greatest abundance was used. As a rule this was the streptococcus which was found in a majority of instances to be of the non-hemolytic type (*Streptococcus salivarius*). In a few instances a predominant growth of *Staphylococcus aureus* was obtained and once the Klebs-Loeffler bacillus outgrew the other varieties.

Rabbits were used exclusively, usually young females weighing from 1,200 to 1,800 gm.

All tissues were fixed for routine examination in Orth's solution, and in formalin for frozen sections and for sections to be stained for bacteria

[1] Here and elsewhere in this paper the term bacteriolysis is used to imply the proteolysis that is assumed to occur when bacterial antigens are subjected to the action of complement and antibody. Jobling and Petersen have recently shown that the phenomenon may be rather a process of solution than of proteolytic cleavage.

by the Giemsa method. All sections, except the last named, were stained by Van Gieson's method.

Giemsa stains were used on nearly all the kidneys, which were carefully examined, without success, for bacteria.

The results of the experiments are given in Table I.

In general the glomerular changes were strikingly slight, though a majority showed one or more of the minor changes commonly found in glomerular nephritis, such as swelling of the endothelium, increase of the endothelial nuclei, and small amounts of granular exudate in the capsular space. If the data are examined closely it will be seen that the extent of such changes bears no appreciable relation to the number of injections or the character of the bacteria injected. Lesions are perhaps somewhat more frequent in those animals which died spontaneously than in those which were killed. In the one or two instances where a true albuminous exudate into the capsular space occurred a coincident spontaneous nephritis was observed. This complicates matters and renders generalizations hazardous. Leaving out these cases, we are at the most justified only in saying that bacterial inoculation produced a very mild grade of intraglomerular damage, but nothing comparable to the diffuse extraglomerular nephritis seen in man, with its exudate of fibrin, serum, and cells into the capsular space.

For bacteriolytic experiments, organisms of the typhoid-colon group are, as is well known, much better suited than streptococci, and it was for this reason that the experiments of Series D with *Bacillus coli communior*, which is highly virulent for rabbits, were carried out. In at least three, and probably all, of the animals of this series, a high degree of immunity, measured in terms of agglutinins was induced (Table II). After immunization it was possible to inject such massive doses of bacteria as in untreated animals would cause sudden death. It was assumed on the basis of the modern belief in the unity of the so called sensitizer antibodies (Zinsser) and our agglutination tests that the animals had acquired greatly heightened powers of bacteriolysis and, hence, that the massive inoculations employed were followed by an inundation of the body with split bacterial protein. Indeed, marked prostration followed the injections and was taken as direct evidence of proteotoxic action. The glomerular changes in this series were perhaps slightly

TABLE I.

Renal Changes after Repeated Intravenous Injections of Bacteria.

Rabbit No.	Original weight of animal.	Culture used.	No. of injections.	Amounts of culture injected.	Day of experiment on which injections were given.	Interval between last injection and death.	Died or killed.	Combined weight of kidneys.	Renal changes.
Series A, *Streptococcus hemolyticus*.									
	gm.			*cc.*		*days*		*gm.*	
1	1,400	No. 1	4	1.0, 1.0, 1.0, 1.0	1, 5, 6, 7	2 hrs.	D.	9.9	Moderate increase of endothelial cells, some with pyknotic nuclei. Granular material in capsular space.
2	1,500	" 1	4	2.0, 2.0, 2.0, 2.0	1, 5, 6, 7	10	"	9.6	Slight desquamation of epithelium of Bowman's capsule.
3	1,500	" 1	4	0.05, 0.05, 0.05, 0.05	1, 5, 6, 7	9	"	8.6	Occasional red blood cells in capsular space. Occasional dilated loop in tufts. Changes slight.
4	1,500	" 1	4	0.02, 0.02, 0.02, 0.02	1, 5, 6, 7	5	"	9.0	Slight increase of endothelial cells and in places stratification of parietal layer of Bowman's capsule.
5	1,750	" 1	3	1.0, 1.0, 1.0	1, 2, 3	14	"	12.1	Considerable increase of endothelial cells of tufts. Some granular exudate in capsular space. Some hyaline casts.
6	1,750	" 1	3	2.0, 2.0, 2.0	1, 2, 3	18	"	10.0	A few glomeruli show increased endothelial cells.
7	1,200	" 1	5	10M.,*† 10M., 10M., 100M., $\frac{1}{3}$ Bl.	1, 2, 3, 33, 44	12	K.	10.8	Endothelium of glomeruli slightly swollen in places. No other notable changes.
8	1,800	" 1	3	10M., 10M., 10M.	1, 2, 3	4	D.	15.2	No notable changes.

9	1,600	No. 1	5	10M., 10M., 10M., 100M., $\frac{1}{3}$ Bl.	1, 2, 3, 33, 44	12	K.	12.0	Moderate endothelial swelling. Occasional dilated empty loops.
10	1,750	" 1	5	20M., 40M., 80M., 100M., $\frac{1}{3}$ Bl.	1, 2, 3, 26, 37	12	"	16.8	Moderate endothelial swelling. Occasional empty loops, not dilated.
11	1,700	" 1	5	20M., 40M., 80M., 100M., $\frac{1}{3}$ Bl.	1, 2, 3, 26, 37	12	"	14.5	About same as No. 10.
12	600	" 1	6	$\frac{1}{6}$ Sl., $\frac{1}{8}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{2}$ Sl., $\frac{1}{2}$ Sl.	1, 2, 3, 4, 5, 6	8	D.	4.7	Considerable granular exudate in capsular space.
13	750	" 1	5	$\frac{1}{6}$ Sl., $\frac{1}{8}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{3}$ Bl.	1, 2, 3, 4, 5	12	K.	7.8	Considerable endothelial proliferation and empty loops.
14	750	" 1	7	$\frac{1}{6}$ Sl., $\frac{1}{8}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{4}$ Sl., $\frac{1}{2}$ Sl., $\frac{1}{2}$ Sl., $\frac{1}{3}$ Bl.	1, 2, 3, 4, 5, 6, 7	12	"	10.3	Slight endothelial proliferation and some empty loops.
15	—	" S 1	1	6.0	1	$\frac{1}{2}$	D.	9.5	Leukocytes in glomeruli. Endothelial nuclei stain deeply.
16	1,300	" S 5	2	10.0, 6.0	1, 10	2	"	13.2	Glomeruli enlarged. Abundant granular and reticular exudate in capsular spaces. Endothelial nuclei stain deeply.
17	2,775	" Ch 1	6	5.0, 5.0, 5.0, 5.0, 5.0, 6.0	1, 6, 12, 19, 27, 33	11	K.	15.3	Moderate swelling and nuclear increase of glomerular endothelium. Yellowish pigment granules in epithelium and lumen of distal convoluted tubules.
18	3,425	" S 16	6	5.0, 5.0, 5.0, 5.0, 5.0, 6.0	1, 6, 13, 19, 27, 33	11	"	24.0	Marked swelling and nuclear increase of glomerular endothelium. Leukocytes in tufts increased. Many hyaline casts.
19	2,100	" S 17	6	5.0, 5.0, 5.0, 5.0, 5.0, 6.0	1, 6, 12, 19, 27, 33	11	"	15.4	Moderate swelling and nuclear increase of glomerular endothelium.
20	1,425	" N 1	7	6.0, 6.0, 6.0, 5.0, 5.0, 5.0, 6.0	1, 38, 44, 50, 57, 65, 71	11	"	—	Practically normal.

*Killed by heat (60°C., 1 hour).

†M. indicates million bacteria; Bl., Blake bottle, agar culture; Sl., agar slant culture. All doses are given in cc. of a 24 hour broth culture, unless otherwise specified.

TABLE I—*Continued.*

Rabbit No.	Original weight of animal.	Culture used.	No. of injections.	Amounts of culture injected.	Day of experiment on which injections were given.	Interval between last injection and death.	Died or killed.	Combined weight of Kidneys.	Renal changes.
				Series B, *Streptococcus viridans.*					
	gm.			*cc.*		*days*		*gm.*	
21	1,450	No. S 6	3	14.0, 5.0, 5.0	1, 11, 38	5	D.	12.2	Glomeruli swollen and bloodless. Capsular space obliterated. Endothelial nuclei increased in places.
22	1,450	" S 7	1	6.0	1	12	"	—	Practically normal.
23	1,540	" S 9	2	6.0, 6.0	1, 28	4	"	10.5	No notable changes.
24	2,900	" S 6	6	6.0, 5.0, 6.0, 5.0, 5.0, 6.0	1, 6, 12, 19, 27, 33	11	K.	16.3	" " "
				Series C, *Staphylococcus aureus.*					
25	2,050	No. S 8	1	6.0	1	1	D.	19.5	Glomeruli large. Endothelium markedly swollen. Occasional leukocytes in capsular space. Many hyaline casts.
26	2,100	" S 10	1	6.0	1	1	"	17.2	No notable changes except spontaneous nephritis.
27	2,700	" S 10	5	2.0, 5.0, 5.0, 3.0, 3.0	1, 7, 12, 19, 34	10	K.	15.5	Glomeruli greatly congested, but endothelium not swollen. Nuclei somewhat increased. Occasional red blood cells in capsular space.
28	2,700	" S 12	6	2.0, 5.0, 5.0, 3.0, 3.0, 3.0	1, 7, 12, 19, 27, 34	10	"	14.2	No notable changes.
29	2,725	" S 10	6	2.0, 5.0, 3.0, 3.0, 3.0, 3.0	1, 7, 12, 19, 27, 34	10	"	16.8	Moderate increase of endothelial nuclei. No other notable changes.

Series D, *B. coli communior*.

30	1,700	*B. c. c.*	5	10M., 10M., 10M., 100M., 1.0	1, 2, 3, 33, 44	12	K.	15.4	Dilated empty loops and swollen epithelium in many tufts. Occasional pyknotic nuclei. Considerable granular exudate in capsular space.
31	1,500	"	5	10M., 10M., 10M., 100M., 1.0	1, 2, 3, 33, 44	12	"	10.7	Moderate swelling and multiplication of endothelium. Occasional dilated empty loops.
32	1,600	"	4	20M., 40M., 80M., 100M.	1, 2, 3, 26	10	D.	13.1	Marked increase of endothelial nuclei. No other marked change.
33	1,600	"	5	20M., 40M., 80M., 100M., 1.0	1, 2, 3, 26, 37	12	K.	12.2	Many empty loops but no marked endothelial swelling.
34	1,250	"	1	$\frac{1}{100}$ Bl.	1	2	D.	—	General capillary and venous engorgement. Occasional red blood cells in capsular space. A few hyaline casts.
35	1,600	"	10	$\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{3}$ Sl., $\frac{2}{3}$ Sl., $\frac{2}{3}$ Sl., 1 Sl., 2 Sl., $\frac{1}{3}$ Bl., $\frac{1}{3}$ Bl.	1, 6, 12, 20, 21, 22, 23, 24, 25, 27	3	"	13.0	Very marked spontaneous nephritis. In fibrotic areas, many of the capsular spaces of the glomeruli contain yellow-staining albuminous exudate, and several others are greatly dilated and contain a non-staining homogeneous exudate. Most of the glomeruli are enlarged, bloodless, with swollen epithelium. Many eosinophils in glomerular and intertubular capillaries. No bacteria found.
36	1,060	"	7	$\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{3}$ Sl., $\frac{2}{3}$ Sl., $\frac{2}{3}$ Sl., 1 Sl.	1, 6, 12, 20, 21, 22, 23	1	"	12.4	Glomeruli enlarged with many dilated empty loops. Endothelium swollen. Some pyknotic nuclei. No exudate in capsular spaces.
37	1,460	"	9	$\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{100}$ Bl., $\frac{1}{3}$ Sl., $\frac{2}{3}$ Sl., $\frac{2}{3}$ Sl., 1 Sl., 2 Sl., $\frac{1}{3}$ Sl.	1, 6, 12, 20, 21, 22, 23, 24, 25	1	"	14.1	Glomeruli moderately enlarged. Slight granular exudate occasionally found in capsular spaces but no marked or extensive glomerular changes.

more marked than in the series in which streptococci and staphylococci were used, but again they failed to correspond in intensity with the number or the amounts of the injections. Thus, Rabbit 37, which received six large injections, suffered but a small amount of glomerular injury. On the whole, it is difficult in our experiments to find support for the bacteriolytic theory of glomerulonephritis.

The fate of bacteria in the kidney is a matter of some interest, but could not be fully investigated in the present work. However, a few observations bearing on this point were made in connection with experiments, previously reported, when successive injections of diphtheria toxin and *Bacillus coli communior* were given. Large amounts of bacteria injected intravenously led to rapid death of the animal. The bacilli were found in dense clumps (Fig. 1), mostly in the glomeruli and

TABLE II.

Agglutination Tested for Rabbits 35, 36, and 37 on the 17th Day after the First Injection.

Rabbit No.	Dilution.										
	1:25	1:50	1:100	1:250	1:500	1:1,000	1:2,500	1:5,000	1:10,000	1:25,000	1:50,000
35	±	+	+	++	+++	+++	++	+	+	±	0
36	+	+	+++	++	+++	+++	+	++	±	++	+++
37	+	+	+	++	++	+++	++	++	+	++	+

occasionally in the intertubular capillaries of the cortex and medulla. In these animals few leukocytes and little evidence of inflammatory reaction were found in the tufts. In animals dying 12 to 48 hours after inoculation the bacteria had usually disappeared and leukocytes, generally in large numbers, were found in the loops. In most of these animals there was a rather marked inflammatory reaction in the glomeruli. It was at first thought that a local bacteriolysis, by leukocytes, with the formation locally of poisonous bacterial disintegration products might be the cause of the inflammatory reaction, but against this was the fact that even with enormous doses (sediment of 30 cc. of broth culture) only a few glomeruli contained demonstrable organisms, whereas the inflammatory reaction involved a majority of the tufts. It was therefore assumed that the toxic substances with their diffuse action,

were circulating in the blood. It does, however, seem fairly probable that the bacteria were occluded and removed by leukocytes, and in one instance this was directly observed (Fig. 2).

The theories which we are examining are closely dependent upon an assumed bacteriolysis of streptococci and it is necessary to examine closely the question whether lysis of the streptococcus can experimentally be shown to occur. To such physical and chemical procedures as freezing and thawing, and extraction with distilled water and weak alkali the streptococcus has, in my hands, proved to be extraordinarily resistant and experiments to be described below indicate that it is correspondingly resistant also to lysis by the Pfeiffer procedure, and by immune serum and complement. The contrast in these respects with the typhoid-colon group is, in fact, so great as to suggest a division of bacteria into what might be called lysostable and lysolabile groups.

In a previous paper (Faber) on experimental arthritis in rabbits it may be recalled that injection of streptococci into the knee-joints of rabbits was followed by a condition designated as sensitization, which was revealed by the occurrence of a local reaction when homologous organisms were later injected intravenously. The term sensitization was purposely chosen to indicate altered properties of the cells of the capsular synovium, without offering a more explicit interpretation of the exact process, whether it was bacteriolysis with the local formation of toxic bacterial disruption products, or an increase in the normal defensive leukocyte-attracting properties of the cells, or a process related in some way to a disturbed cellular ferment balance consequent to local specific antibody-antigen combinations. In point of fact, certain unpublished experiments were carried out at that time in an attempt to elucidate the problem in one of these directions, and may be cited here.

Pfeiffer's Phenomenon for Joints.

Rabbit 40.—Left knee injected with mixture of 0.1 cc. of immune serum + 1 loop of *Streptococcus viridans* + 0.9 cc. of 0.85 per cent salt solution.

Right knee injected with mixture of 1 cc. of sterile bouillon + 1 loop of *Streptococcus viridans*.

Both knees aspirated after 1 hour. Cultures: left knee, heavy growth of streptococcus; right knee, no growth.

Rabbit 41.—Left knee injected with mixture of 0.1 cc. of normal rabbit serum + 0.9 cc. of salt solution + 1 loop of *Streptococcus viridans*.

Right knee injected with mixture of 1 cc. of bouillon + 1 loop of *Streptococcus viridans*.

Both knees aspirated after 1 hour. Both cultures sterile.

The immune serum was obtained from a rabbit which had received two injections of living streptococcus. Complement fixation was positive in the serum. This rabbit developed an acute arthritis immediately after receiving a third injection (shortly after the time that blood for the above serum was drawn).

In connection with the above experiments, the following cultural tests were made.

(*a*) 1 loop of *Streptococcus viridans* + 1 cc. of immune serum. Incubated 1 hour and plated. Result scanty growth.

(*b*) 1 loop of *Streptococcus viridans* + 1 cc. of normal serum. Incubated 1 hour and plated. Result heavy growth.

(*c*) 1 loop of *Streptococcus viridans* plated. Result heavy growth.

These experiments, though too few to be decisive, suggest the following conclusions. (1) Immune streptococcus serum not only fails to evoke the Pfeiffer phenomenon, but may even favor the bacteria at the expense of the tissues. (2) The normal synovial membrane has considerable power to combat infection. (3) Immune serum apparently has the power of partially inhibiting growth *in vitro*.

The third point might be used as an indication of bacteriolysis and was therefore tested more completely in another series of experiments which are given in Table III.

The following points may be emphasized. (1) The lowest counts were obtained in those plates in which the dilution of the serum had been respectively 1:30 and 1:50. The maximum agglutination was obtained with a dilution of 1:40. (2) The lowering of the bacterial count was independent of the amount of complement added. (3) Lowering of the bacterial count was obtained without complement and in two instances was greater than in any of the tubes to which complement had been added.

These experiments strongly suggest that we are dealing not with bacteriolysis proper but with an agglutination phenomenon. It is

obvious that an agglutinated clump of bacteria will give but a single colony and that agglutination in this way will cause an apparent reduction of the bacterial count when the pour-plate method is used.

TABLE III.

Tests for Bacteriolysis with Streptococcus viridans, Complement, and Immune Serum.

Complement.	Row.	Cc. of immune serum.						
		0.1	0.08	0.05	0.03	0.01	0.007	0.00
		Dilution.						
		1:15	1:20	1:30	1:50	1:150	1:200	—
		Tube.						
		1	2	3	4	5	6	7
cc.								
0.5	A	180	150	125	180	225	180	300
0.3	B	170	250	105	110	200	180	
0.1	C	160	160	75	40	140	160	
0.07	D	125	150	90	150	150	175	
0.04	E	90	150	110	19	100	110	
0.00	F	160						375

The figures represent thousand colonies per plate.

To each tube was added 0.5 cc. of a suspension of streptococci containing 750,000 bacteria per cc. All the tubes before incubation were made up to equal volume with 0.85 per cent sodium chloride solution.

DISCUSSION.

It may be assumed that when bacteria are introduced into a tissue the mechanism of defense is one of physical removal, depending on the leukotactic activity of the tissue or bacteria in question (Fig. 2). It may also be assumed that this activity is heightened by previous exposure of the tissue to the infecting agent and that the reaction will usually be accompanied by such other reactive phenomena as vasodilation and exudation of fluid. Bacteriolysis of streptococci *in situ* and the local production of toxic disintegration products is at the least an improbable explanation of the phenomenon.

The conclusions to be derived from the experiments on joints may perhaps fairly be applied to the kidney, save that in the latter we have obtained much less evidence of reactivity. It is evident that the

arrangement of the glomerular capillaries is such that small bacterial emboli can be expeditiously removed by phagocytes and that there is in the tuft no closed space in which inflammatory exudate can collect. It may, indeed, be doubted whether these emboli are permitted to remain long enough in contact with the glomerular endothelium to set up a state of sensitization. In none of the rabbits examined were bacterial emboli found more than 12 hours after injection and then in only one instance when the animal had been profoundly intoxicated with diphtheria toxin.

The capsular space, on the other hand, does supply conditions of relatively poor drainage which are increased when the excretion of water from the tuft is interfered with. Apparently, it is when the lining epithelium of Bowman's capsule has been damaged and permits toxic substances to pass through that leukocytes, fibrin, and the other materials of inflammatory exudate are attracted to the capsular space and so produce the picture of extraglomerular nephritis. This picture, it will be recalled, was produced when an injection of bacteria followed injury by diphtheria toxin. The present series of experiments with their consistently negative results constitutes strong evidence that bacteria alone are unable to produce extraglomerular nephritis, even by cumulative action or by immune bacteriolysis, since on the one hand a bacterial species known to be subject to immune bacteriolysis failed to produce the lesions under favorable conditions, and since on the other hand the organism commonly found in this disease has been shown to be extraordinarily resistant to bacteriolysis.

The evidence of the present experiments and the arguments presented against the bacteriolytic theory would also appear to apply equally well to the assumption of a local allergic reaction in the glomerular endothelium.

Embolism, even in an immune host where agglutination *in vivo* may be assumed to occur, is too sporadic in the kidney to explain lesions as widespread and evenly distributed as occur in the human disease, and it seems more than ever necessary to assume the action of a soluble poison in the blood as the immediate pathogenetic factor. For the present there is no direct proof of the nature or of the origin of this poison.

The local concentration of poisons in the tuft, whatever may be their source or nature, may, however, still be held to be the immediate cause for the occurrence of lesions at this point.

SUMMARY.

Repeated injections into the blood stream of streptococci and staphylococci derived from cases of scarlet fever, and of *Bacillus coli communior* failed to produce typical glomerulonephritis even when immune antibodies could be demonstrated in the serum in high dilutions. Bacteriolysis of streptococci was not found by the usual tests *in vitro* or by the Pfeiffer procedure. It is therefore concluded that the weight of evidence is against the theory that glomerulonephritis is due to immune bacteriolysis of streptococci. The experiments also failed to give any support to the hypothesis of allergy or of sensitization as a factor in the production of the disease. Evidence is presented to show that bacterial emboli are rapidly removed from the glomerular capillaries by leukocytes, and that this embolism, even after injections of enormous quantities of bacteria, affects but a small proportion of the glomeruli. It is again suggested that a circulating poison in the soluble state is responsible directly for the disease in question.

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EXPLANATION OF PLATE 54.

FIG. 1. Rabbit 38. Intravenous injection of the sediment of 30 cc. of a broth culture of *B. coli communior*. Died 1 hour later. Large bacterial embolus in glomerulus and its afferent vessel. Giemsa stain. Leitz obj. 6.

FIG. 2. Rabbit 39. Intravenous injection of one agar slant of *B. coli communior*. Died 12 hours later. Bacterial embolus in glomerulus. Adjacent to this is a polymorphonuclear leukocyte containing a bacillus. Giemsa stain. Obj. oil immersion.

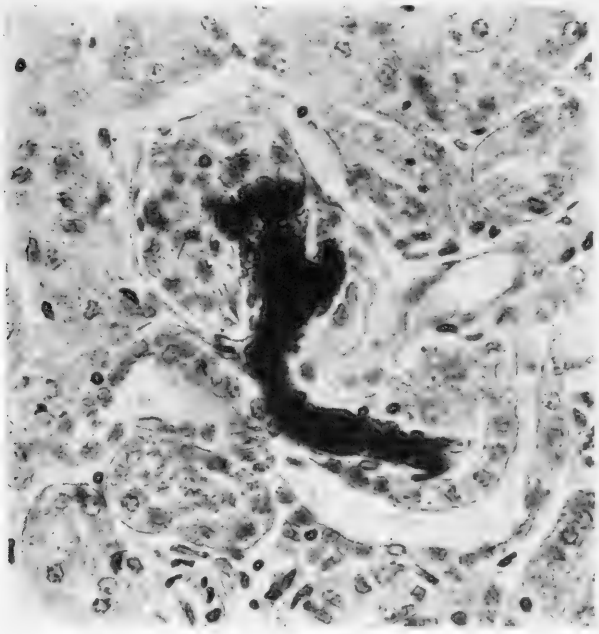

FIG. 1.

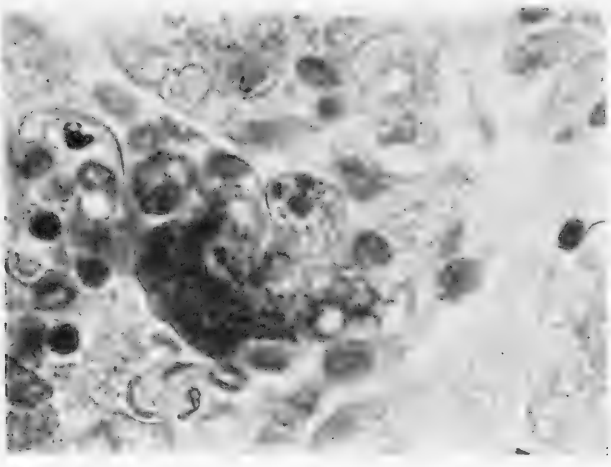

FIG. 2.

(Faber and Murray: Studies in glomerulonephritis. III.)

THE INTERRELATION OF THE SURVIVING HEART AND PANCREAS OF THE DOG IN SUGAR METABOLISM.

Second Paper.

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The clue which led to the present investigation was the discovery that when a Locke's solution containing dextrose was perfused through the pancreas of a dog, there was a distinct diminution in the optical rotation of the perfusate but no change in its reducing properties. This result, which I had not anticipated, seemed of interest, for it would indicate that the perfused pancreas exerts an independent effect on dextrose, a fact which had not been evident from the reducing properties alone. The following experiments were carried out in an attempt to analyze the nature of this effect and to study further the general problem of the interrelation between the heart and pancreas in sugar metabolism with especial relation to their specific action, separately and combined, on dextrose and levulose. The results have been definite and have led to a tentative hypothesis which at present seems best suited to explain the facts that have been obtained.

In the previous investigation (1) it was found when a pancreas was perfused aseptically with Locke's solutions containing physiological concentrations of dextrose that, while there was no change in the reducing properties of the perfusate, yet the pancreas supplied something to the perfusate which subsequently brought about a utilization of sugar by the living heart to an extent that did not occur with the heart alone. This pancreatic substance, as far as could be determined, possessed the characteristics of an enzyme. It was inactivated by boiling; it acted in small amounts; it was unstable, rapidly becoming inactive on standing; it caused a great acceleration in the rate of a reaction which otherwise proceeded slowly, and the rate of reaction diminished as the reaction proceeded. The living heart in the presence of this pancreatic factor was responsible for two effects: first, a change of the sugar to a non-reducing form that yielded again a simple sugar on hydrolysis or by simply standing with a preservative at 37°C. for 24 hours; second, a disappearance of sugar which was probably due to its destruction by hydrolysis or oxidation.

Methods.

Briefly outlined, the methods of approaching the problems in the present work were by perfusing the living heart and pancreas with oxygenated Locke's solutions containing dextrose and levulose. With these factors, the actual combinations which were used are given below:

(*a*) Pancreas + dextrose.
(*b*) Pancreas + levulose.
(*c*) Pancreas + dextrose + levulose.
(*d*) Heart + dextrose.
(*e*) Heart + levulose.
(*f*) Heart + dextrose + levulose.
(*g*) Pancreas + heart + dextrose.
(*h*) Pancreas + heart + levulose.
(*i*) Pancreas + heart + dextrose + levulose.

With these tissue and sugar combinations, the original Locke's solutions were compared with the final perfusates, both before and after hydrolysis, as to their optical rotation, reducing power, and, in a number of experiments, the melting points of the osazones.

Perfusions.—The same method of isolated perfusion and Locke's solutions of the same composition were used as in the former experiments (1), and the same aseptic technique and standard of asepsis were rigidly maintained. No heart perfusion was considered in this series where the heart did not continue to beat actively over the period of perfusion. The bacterial counts given in the tables show that exclusion of bacteria was even more successful than formerly.

Sugars.—The sugars were dextrose (Kahlbaum, pure) and levulose (Kahlbaum, pure, from inulin). In a few of the early experiments, Schering's crystalline powdered levulose was used, but as it showed no difference in its action from the Kahlbaum levulose which was subsequently obtained, the experiments are included. The sugar in each experiment was dissolved and boiled for at least 10 minutes before adding it to the Locke's solution, to eliminate any phenomenon of mutarotation. With an occasional exception the concentrations of sugar in the original Locke's solutions were from 0.3 to 0.4 per cent.

Sugar Determinations.—The quantitative sugar determinations were made both by the colorimetric method of Lewis and Benedict (2) and with the polariscope. The preliminary procedure was as follows: 50 cc. each of the original Locke's solutions and of the perfusates were measured in volumetric flasks and each portion was

added to 1 gm. of trichloracetic acid carefully weighed from the same stock bottle. This amount of reagent gave a complete precipitation of whatever protein was present in the perfusates, and on filtration yielded a clear fluid. To each filtrate sufficient concentrated hydrochloric acid was added to give the desired per cent of acidity. As far as could be ascertained, a hydrolytic effect was obtained with dextrose solutions when either 1 per cent or 0.5 per cent hydrochloric acid was used, while with levulose 0.5 per cent hydrochloric acid could not be exceeded without sugar destruction. From these acidified specimens samples were immediately taken for sugar determination. The samples taken for the polariscope were returned and the solutions placed in a boiling water bath for an hour with reflux condensers. At the end of this time they were cooled and specimens were again removed for determinations of optical rotation and reduction. In each experiment the amount of reducing sugar was based on the polariscopic determination of the original Locke's solution. By this procedure the reducing sugar and optical rotation were measured before and after hydrolysis in the original Locke's solution and in the perfusates under constant conditions of volume and acidity.

The possibility was suggested at first that in precipitating the protein a portion of the sugar might be carried down. In control experiments, however, as shown in the tables, this did not occur. In these controls even with the precipitation of a relatively large amount of protein the filtrate did not appreciably vary from the original Locke's solution, either in rotation or in reduction. The addition of trichloracetic acid and hydrochloric acid increased the volume of fluid slightly and so diminished the sugar per cent. However, since this increase in volume is constant for all samples, the significance of comparative determinations remained unchanged.

The polariscopic determinations were made with a Schmidt and Haensch polariscope which gives a direct reading to the hundredth of a degree. In each experiment all readings were made in a 4 dm. tube under constant temperature conditions, and the final reading was taken as the average of five successive determinations not varying over 0.02°. This gave results which could be compared with a fair degree of accuracy to the third decimal place. The specific

rotation of dextrose was taken as +52.5°, and of levulose as −91°. The controls gave a final check on the accuracy of the results.

TABLE I.

Hydrogen Ion Concentrations of Perfusates.

Experiment No.	Tissues perfused.	Duration of perfusion.	pH Locke's solution.		pH perfusate.		Sugar used
			Carbon dioxide present.	Carbon dioxide removed.	Carbon dioxide present.	Carbon dioxide removed.	
		hrs.					
1	Pancreas + heart.	4¾	7.8	8.0	7.2	7.9	Dextrose.
2	" + "	5	7.9	8.0	6.8	7.6	"
3	" + "	5	7.7	7.9	6.8	7.4	"
4	Heart.	4	7.9	8.0	6.8	7.6	"
5	"	4	8.0	8.1	6.8	7.5	"
6	"	4	8.1	8.2	6.9	7.7	"
7	"	4	8.2	8.3	7.4	7.9	Levulose.
8	"	4	8.0	8.1	7.4	7.9	"
9	"	4	7.9	8.0	7.35	7.9	"
10	"	4	8.0	8.1	7.4	7.9	"
11	Pancreas + heart.	5	7.9	8.1	7.5	7.8	"
12	" + "	5	8.0	8.1	7.45	8.0	"
13	" + "	5	7.9	8.1	7.3	7.9	"
14	Heart.	4	8.0	8.1	7.5	7.9	Dextrose+ levulose.
15	"	4	8.1	8.2	7.8	7.9	Dextrose+ levulose.
16	"	4	8.1	8.2	7.5	7.8	Dextrose+ levulose.
17	Pancreas + heart.	5	8.0	8.2	7.5	7.9	Dextrose+ levulose.
18	" + "	5	8.0	8.15	7.4	7.9	Dextrose+ levulose.
19	" + "	5	8.0	8.1	7.7	8.1	Dextrose+ levulose.
20	" + "	5	8.0	8.2	7.55	7.9	Dextrose+ levulose.
21	Pancreas + non-beating heart (control).	5	8.0	8.1	7.45	8.05	Dextrose+ levulose.
22	Pancreas.	1½	8.0	8.1	7.8	8.0	Dextrose.
23	"	4	8.1	8.2	7.8	8.0	Dextrose+ levulose.
24	"	5	8.0	8.1	7.9	8.0	Dextrose.

Controls.—The following controls were used:

(*a*) In each experiment a sample of the original Locke's solution was subjected to the same procedure as the perfusate except for the actual circulation through the tissues. In this respect only the sample

TABLE II.

Effect of the Locke's Solutions Used on the Optical Rotation of Dextrose and Levulose.

No. of experiments averaged.	Sugar in the Locke's solution.	Method of treatment.		Duration of experiment.	Optical rotation with 4 dm. tube.					
					At beginning of period.		At end of period.		Change in rotation.	
				hrs.		*per cent*		*per cent*		*per cent*
4	Dextrose.	Incubation, 3 experiments. Perfusion, 1 experiment.	Maximum.	24	+1.105°	0.526	+1.104°	0.525	+0.014°	0.0066
			Minimum.	4	+0.408°	0.194	+0.406°	0.193	−0.001°	0.0009
			Average.	9	+0.784°	0.373	+0.786°	0.374	+0.007°	0.0033
3	Levulose.	Incubation, 2 experiments. Perfusion, 1 experiment.	Maximum.	24	−1.572°	0.431	−1.572°	0.431	+0.004°	0.0019
			Minimum.	4	−1.313°	0.360	−1.310°	0.359	0.000°	0.0000
			Average.	10.6	−1.411°	0.387	−1.412°	0.387	+0.001°	0.0005
2	Dextrose+ levulose.	Incubation, 2 experiments.	Maximum.	4	−0.792°		−0.792°		−0.006°	
			Minimum.	2	−0.726°		−0.720°		0.000°	
			Average.	3	−0.759°		−0.756°		−0.003°	

Average change in rotation in nine experiments in average of 7.5 hours = + 0.0016°.

of Locke's solution differed from the perfusate. This gave a fair basis for comparing the Locke's solutions and the perfusates.

(*b*) In a number of those experiments in which the pancreas was perfused alone, the unperfused splenic portion was macerated and ground aseptically with sand and about the same relative amount of Locke's solution as was perfused through the main portion of the

pancreas. This controlled the question whether an extract of the pancreas is similar in its effect to a perfusate.

(*c*) As enzyme action in general is sensitive to hydrogen ion concentration, the reactions of the Locke's solutions and perfusates were measured, in the majority of experiments, by the colorimetric method of Levy, Rowntree, and Marriott (3). In Table I are given examples of the variations which occurred. While there was a certain variation both before and after removal of carbon dioxide, yet this variation was practically within the normal body limits and no effects were obtained which would indicate that a relation existed between the reaction of the perfusate and the experimental results.

(*d*) In Table II are given the results of nine experiments which show that when specimens of Locke's solution similar to those used in the perfusion experiments were incubated or perfused in the apparatus for varying periods of time, there is no essential variation in the optical rotation when either dextrose, levulose, or a mixture of the two is present. In the nine experiments with an average duration of 7.5 hours there was a maximum change in the rotation of 0.014° and a minimum change of 0.000°. The average change was an increased positive rotation of 0.0016°. These measurements were made in the 4 dm. tube used throughout this work.

RESULTS.

Pancreas Perfusions.

Eleven experiments were carried out in which a pancreas was perfused with an oxygenated Locke's solution containing dextrose. The final perfusates consistently showed a diminished optical rotation as compared with the Locke's solutions, but no change in the amount of reduction. The perfusates in four experiments were then hydrolyzed with the consistent result, also, that there was a partial restoration of the rotation to the original level, but no change in the reducing properties. These changes were considerably greater than any possible error in the polariscopic or reducing methods, and were controlled not only by the samples of Locke's solution subjected to an analogous procedure, but also, in five experiments, by the effect of extracts of a portion of the same pancreas on the same original

Locke's solution. The results are summarized in Table III and charted in Text-fig. 1. In Text-fig. 2 an experiment is given in which successive readings were made showing the curve of diminution in the optical rotation of a perfusate containing approximately 0.4 per cent of dextrose.

The question then arose: Was this phenomenon dependent upon a direct contact of the dextrose with the pancreatic tissue? Former experiments had shown that the rapid utilization of dextrose by the heart took place if dextrose was merely added to a pancreatic perfusate before being fed to the heart. In the three experiments shown in Table III and Text-fig. 1, the pancreas was first perfused with a Locke's solution containing no dextrose, and then dextrose was added to the perfusate and the solution allowed to incubate. As illustrated in Text-fig. 1, the change in rotation per unit of time was almost identical with the change which occurs when the pancreas is directly perfused with dextrose. This would indicate, therefore, that direct contact between the dextrose and pancreatic tissue is not necessary but that the change can be brought about by some substance washed out of the perfused gland.

Is this effect specific for dextrose in any way analogous to the specificity of most enzyme actions? Levulose was selected as a sugar for comparison. In six experiments, as shown in Table III and Text-fig. 1, the pancreas was perfused for an average period of 3 hours. In no experiment was there the least detectable change in the optical rotation or reducing properties of the sugar beyond the limits of experimental error. Hydrolysis of the final perfusates gave practically no change in the optical rotation or reduction. In four experiments, also, a pancreatic extract showed no effect. It seemed evident, therefore, that the action of the pancreas is specific for dextrose.

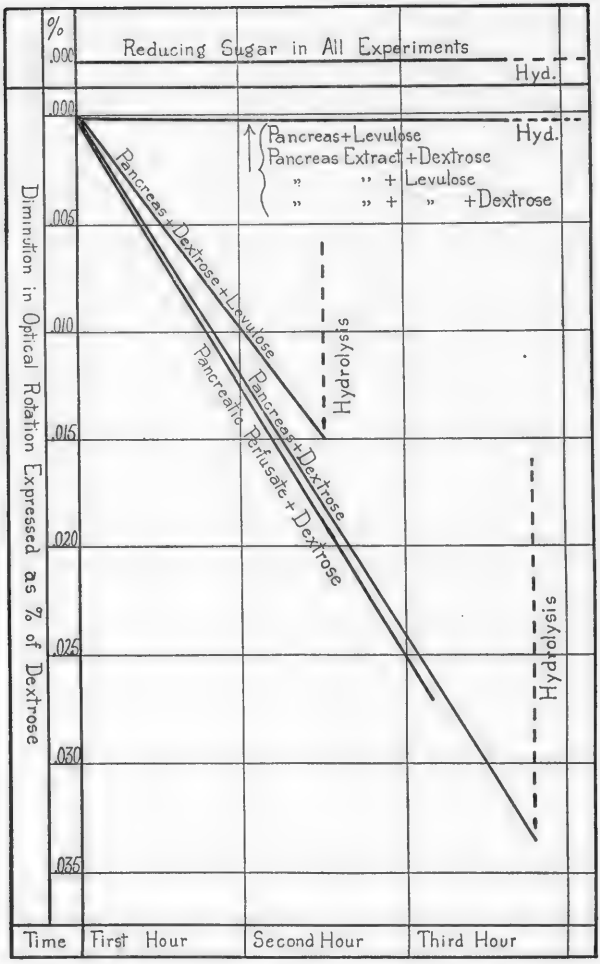
A further method for testing this specificity suggested itself. If a Locke's solution containing dextrose and levulose is used, there is a certain initial balance of rotation between the two sugars. If the effect of the pancreas is specific in diminishing the positive rotation of a dextrose solution, then, by selecting the dextrose from a mixture of the two sugars, there should be a shifting of the balance toward the negative side. This is what occurred in six experiments which were successfully carried out. The negative rotation was always

TABLE III.

Experiments Showing the Effect of the Perfused Pancreas and of Pancreas Extracts on the Reducing Properties and Optical Rotations of Dextrose and Levulose.

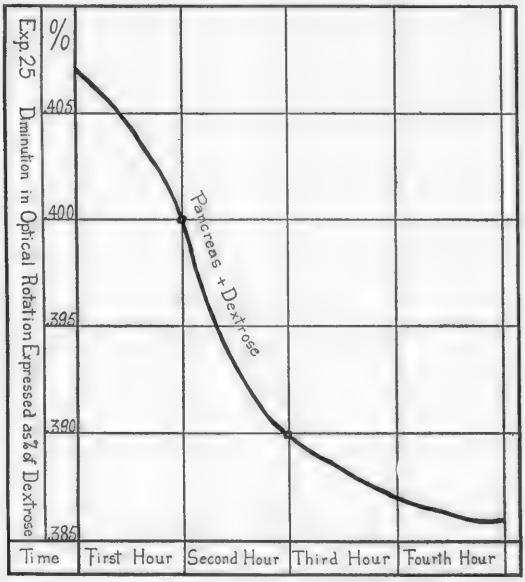
No. of experiments averaged.	Sugar used.	Type of perfusion.		Duration of perfusion.	Optical rotation with 4 dm. tube.								Difference in reducing sugar between Locke's solution and perfusate.	Bacteria per cc. in final perfusate.
					Before hydrolysis.					After hydrolysis.				
					Locke's solution.		Final perfusate.			No. of experiments hydrolyzed.	Change in optical rotation estimated as dextrose.			
											Locke's solution.		Final perfusate.	
									Diminished per cent of perfusate as dextrose.					
				hrs.		*per cent*		*per cent*	*per cent*			*per cent*		*per cent*	*per cent*	
11	Dextrose.	Pancreas.	Maximum.	5	+0.880°	0.419	+0.838°	0.399		4	+0.002°	+0.0009	+0.046°	+0.022	0.002	4,000
			Minimum.	0.6	+0.408°	0.194	+0.344°	0.163			0.000°	0.000	+0.016°	+0.007	0.000	2
			Average.	2.8	+0.775°	0.369	+0.707°	0.336	0.033		+0.001°	+0.0004	+0.036°	+0.017	0.0007	700
6	Levulose.	Pancreas.	Maximum.	4	−1.404°	0.386	−1.402°	0.385		3	0.000°	0.000	0.000°	0.000	0.001	1,800
			Minimum.	1	−0.678°	0.186	−0.675°	0.185			0.000°	0.000	0.000°	0.000	0.000	6
			Average.	3	−1.200°	0.329	−1.199°	0.329	0.0003		0.000°	0.000	0.000°	0.000	0.0005	1,000
6	Dextrose + levulose.	Pancreas.	Maximum.	4	−0.292°	—	−0.324°	—		4	+0.002°	+0.0009	+0.036°	+0.017	0.001	900
			Minimum.	1	−0.182°	—	−0.258°	—			0.000°	0.000	+0.020°	+0.009	0.000	3
			Average.	1.5	−0.267°	—	−0.300°	—	0.015		+0.0005°	+0.0002	+0.019°	+0.009	0.0002	200
5	Dextrose.	Pancreas extract.	Maximum.	4	+1.104°	0.525	+1.104°	0.525							0.000	800,000
			Minimum.	2	+0.408°	0.194	+0.408°	0.194							0.000	240
			Average.	3.6	+0.681°	0.324	+0.680°	0.323	0.0003						0.000	268,000

4	Levulose.	Pancreas extract.	Maximum.	4	−1.574°	0.432	−1.574°	0.432							0.001	3,000
			Minimum.	4	−0.678°	0.186	−0.676°	0.185							0.000	200
			Average.	4	−1.145°	0.314	−1.144°	0.314	0.0003						0.0005	1,700
2	Dextrose + levulose.	Pancreas extract.	Maximum.	4	−0.792°	—	−0.792°	—							0.001	250
			Minimum.	2	−0.720°	—	−0.720°	—							0.000	36
			Average.	3	−0.756°	—	−0.756°	—	0.000						0.0005	150
3	Dextrose.	Pancreas perfusate.	Maximum.	4	+1.106°	0.526	+1.050°	0.500							0.001	3,100
			Minimum.	1.5	+0.380°	0.181	+0.350°	0.166							0.000	2,000
			Average.	3.2	+0.721°	0.343	+0.665°	0.316	0.027						0.0007	2,600
4	Dextrose + levulose.	Spleen.	Maximum.	1	−0.314°	—	−0.314°	—		4	+0.004°	+0.0019	−0.014°	−0.006	0.002	140
			Minimum.	0.75	−0.292°	—	−0.292°	—			−0.014°	−0.006	0.000°	0.000	0.000	0
			Average.	0.88	−0.300°	—	−0.300°	—	0.000		−0.014°	−0.006	−0.008°	−0.004	0.0007	56



TEXT-FIG. 1. Chart of Table III representing forty-one experiments. The chart shows that when the pancreas was perfused with a Locke's solution containing dextrose there was no change in the reducing properties of the perfusate but a distinct diminution in the optical rotation. This also occurred if dextrose was added to a sugarless perfusate after removal of the pancreas from the apparatus. A mixture of dextrose and levulose perfused through the pancreas gave an increased negative rotation indicating a diminution of the dextrose effect. On hydrolysis of these perfusates there was a partial restoration of the optical rotation. These changes did not occur if the pancreas was perfused with levulose nor did a fresh pancreatic extract have a similar effect on either sugar.

increased as shown in Table III and Text-fig. 1. The change in rotation estimated as dextrose per unit of time was only slightly less than if dextrose alone had been used. Hydrolysis gave a distinct restoration of the original balance. In no instance was there any variation in the reducing properties of the perfusates or Locke's solutions beyond the limits of experimental error.



TEXT-FIG. 2. Experiment 25. Curve showing the rate of diminution in optical rotation of a Locke's solution containing approximately 0.4 per cent of dextrose when perfused 4 hours through a pancreas. The reducing sugar remained unchanged.

These experiments were controlled by the action of pancreas extracts and by four experiments in which the spleen was perfused with a Locke's solution containing dextrose and levulose. In neither case was there any change, either in the optical rotation or in the reducing properties. With mixtures of dextrose and levulose, the conclusion would seem to be that the pancreas has a specific action on dextrose, an action which is not given by the perfused spleen, or by a pancreatic extract.

Heart-Pancreas Perfusions.

It was obvious from previous experiments with dextrose that a characteristic difference exists between a heart perfusate and one which has passed through both the heart and pancreas. Hydrolysis of a heart-pancreas perfusate gives an increase in reducing sugar, while a heart perfusate gives no such increase. The hypothesis suggested to explain this fact was that the heart and pancreas together brought about a condensation of the dextrose to some non-reducing form of polysaccharide.

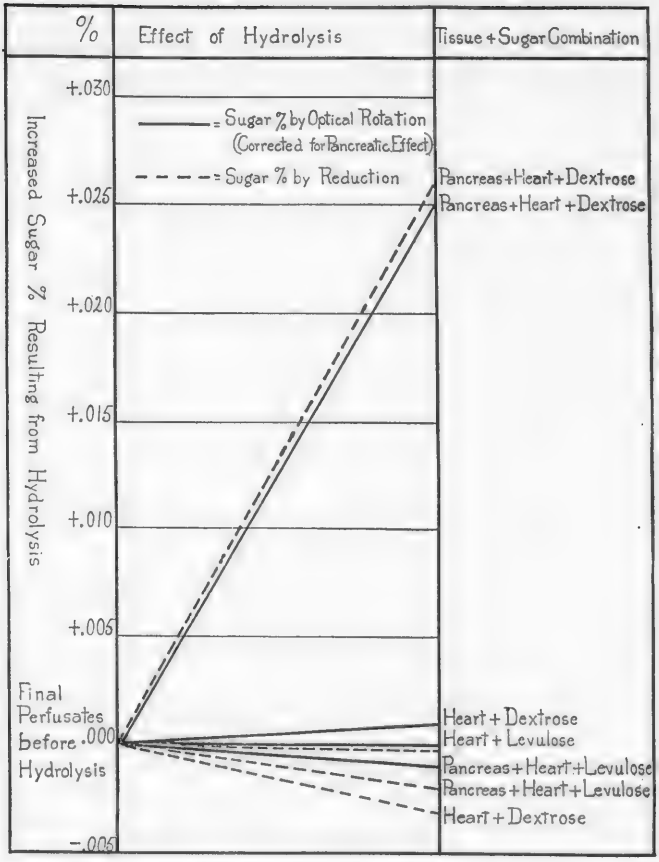
TABLE IV.

Experiments on the Interrelation and Specificity of the Heart and Pancreas in Their Action on Dextrose and Levulose.

No. of experiments averaged.	Sugar used.	Tissues perfused.		Final perfusate. Optical rotation with 4 dm. tube.					Final perfusate; change in reducing sugar on hydrolysis.
				Before hydrolysis.		After hydrolysis.		Change due to pancreas and heart, corrected.*	
					per cent		*per cent*	*per cent*	*per cent*
3	Dextrose.	Heart.	Maximum.	+0.648°	0.308	+0.650°	0.309		
			Minimum.	+0.532°	0.253	+0.534°	0.254		
			Average.	+0.591°	0.281	+0.592°	0.282		−0.0030
3	Levulose.	Heart.	Maximum.	−1.304°	0.358	−1.306°	0.359		
			Minimum.	−1.100°	0.302	−1.100°	0.302		
			Average.	−1.169°	0.321	−1.169°	0.321		0.0000
3	Dextrose.	Pancreas +heart.	Maximum.	+0.654°	0.311	+0.730°	0.347		
			Minimum.	+0.486°	0.231	+0.544°	0.259		
			Average.	+0.576°	0.274	+0.641°	0.305	+0.0250	+0.0260
3	Levulose.	Pancreas +heart.	Maximum.	−1.194°	0.328	−1.188°	0.326		
			Minimum.	−0.966°	0.263	−0.964°	0.262		
			Average.	−1.116°	0.306	−1.113°	0.305	−0.0004	−0.0020

The average amount of perfusate in each experiment was 252 cc.; the duration of each experiment, 4 hours. Bacteria per cc. in final perfusates: maximum, 640; minimum, 5; average, 122.

* Corrected for the preliminary change in optical rotation due to the pancreas alone.



TEXT-FIG. 3. Chart of Table IV. The final perfusates from twelve experiments of different types were hydrolyzed and the reducing properties and optical rotations of the perfusates determined before and after hydrolysis. The percentage increase or decrease in sugar by these two independent methods is represented in this chart. The tissue and sugar combinations are indicated to the right. Correction is made for the previous independent effect of the pancreas on dextrose.

The experiments show the specificity of the heart-pancreas combination in condensing dextrose to a non-reducing substance with an optical rotation less than that of dextrose. This does not occur with the heart alone when perfused with dextrose or with any combination of the heart and pancreas with levulose.

The results of the twelve perfusions which were carried out are summarized in Table IV and shown graphically in Text-fig. 3. In these experiments the reducing properties and optical rotations of the final perfusates were determined before and after hydrolysis. If the optical rotation of a perfusate were altered by hydrolysis it would indicate that some new substance had been formed and whether the optical rotation of this substance were greater or less than the original sugar. A change in the optical rotation and an increase in the amount of reduction on hydrolysis would show that this substance had probably undergone a dissociative change.

The experiments clearly indicate that it is only with the heart-pancreas combinations that dextrose is transformed to a non-reducing substance which has an optical rotation less than that of dextrose. This transformation did not occur when the heart was perfused alone with dextrose nor did it occur with any combination of the heart and pancreas with levulose.

A clear demonstration of this specific action of the pancreas and heart on dextrose was shown in the twelve experiments given in Table V and Text-fig. 4. In these experiments the pancreas, the spleen, or the kidneys were first perfused, and their perfusates compared in their ability to enable the heart to select dextrose from a mixture of dextrose and levulose. A displacement of the balance in optical rotation of a perfusate containing approximately equal parts of these sugars would demonstrate which sugar had been changed or used most rapidly. Hydrolysis of the final perfusates would, further, indicate, by the extent to which the optical rotation was restored, the amount of change which had taken place.

The results of these experiments were definite. When the heart was perfused alone with a Locke's solution containing dextrose and levulose, there was a distinct utilization of the sugars as shown by the change in reduction. The optical rotations of the final perfusates, however, either were unchanged or were slightly increased on the negative side. This gave definite proof that both sugars had been used either in approximately equal amounts or that there had been a slightly greater utilization of dextrose over levulose. If the spleen was first perfused and the perfusate subsequently passed through the heart, the selective action of the heart for dextrose was practically

the same as with the heart alone. Finally, the kidneys were perfused and gave a certain removal of levulose but subsequent perfusion of the fluid through the heart gave a shift in the optical rotation which, once more, was only slightly greater than with the heart alone.

TABLE V.

Experiments on the Specificity of the Pancreas in Enabling the Heart to Select Dextrose from a Mixture of Dextrose and Levulose.

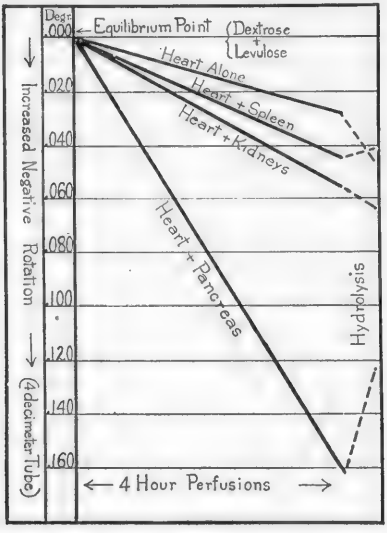
No. of experiments averaged.	Tissues perfused.		Optical rotation with 4 dm. tube.					
			Locke's solution.		Final perfusate.			
			Before hydrolysis.	After hydrolysis.	Before hydrolysis.	Increased negative rotation, corrected.*	After hydrolysis.	Change in rotation after hydrolysis, corrected.*
4	Heart.	Maximum.	−0.308°	−0.306°	−0.350°			Increased negative 0.013°
		Minimum.	−0.280°	−0.264°	−0.286°			
		Average.	−0.295°	−0.285°	−0.323°		−0.326°	
4	Pancreas + heart.	Maximum.	−0.292°	−0.286°	−0.520°			Diminished negative 0.027°
		Minimum.	−0.280°	−0.274°	−0.428°			
		Average.	−0.286°	−0.280°	−0.460°	0.162°	−0.415°	
2	Kidneys + heart.	Maximum.	−0.250°	−0.242°	−0.300°			Increased negative 0.003°
		Minimum.	−0.119°	−0.121°	−0.190°			
		Average.	−0.184°	−0.181°	−0.245°	0.056°	−0.250°	
2	Spleen + heart.	Maximum.	−0.314°	−0.300°	−0.368°			Increased negative 0.004°
		Minimum.	−0.292°	−0.290°	−0.328°			
		Average.	−0.303°	−0.295°	−0.348°	0.045°	−0.344°	

The average amount of perfusate in each experiment was 250 cc.; the duration of each experiment, 4 hours. Bacteria per cc. in final perfusates: maximum, 880; minimum, 0; average, 183.

* Corrected for rotation due to pancreas, kidneys, or spleen alone.

In contrast to these experiments were those in which the pancreas was first perfused and the perfusate subsequently circulated through the heart. Instead of an optical rotation which varied either not at all or only moderately from an equilibrium between the two sugars, there was a striking shift to the left even after correcting for

the preliminary effect of the pancreas. Hydrolysis of the final perfusate, also, diminished the negative rotation to some extent, while



TEXT-FIG. 4. Chart of Table V. Twelve experiments in which the spleen, kidneys, and pancreas were first perfused and their perfusates compared in their ability to enable the heart to select dextrose from a mixture of dextrose and levulose. Displacement of the balance in optical rotation of a perfusate containing equal parts of these sugars would indicate which sugar had been changed or used most rapidly, while hydrolysis of the final perfusates would indicate, by the extent to which the optical rotation was restored, the amount of condensation and of utilization. Correction was made in each experiment for any preliminary effect of the spleen, kidneys, or pancreas.

The experiments indicate that the heart, or the heart plus spleen or plus kidneys over a 4 hour period of perfusion, uses relatively a little more dextrose than levulose. When, however, the heart and pancreas are combined the relative utilization of dextrose by the heart is greatly increased and subsequent hydrolysis of the perfusates restores the optical rotation to some extent, which does not occur when the spleen or kidneys are combined with the heart.

in the previous experiments where the heart had been perfused alone or with the kidneys or spleen, hydrolysis of the final perfusates had slightly increased the negative rotation.

Osazones.

A further possible method of demonstrating the above changes was available in the melting points of the osazones which could be obtained at different stages in various types of experiments when dextrose was used. The following technique was observed: 10 cc. specimens were taken, both of the original Locke's solution and of the protein-free perfusate. They contained the same reagents and differed in their previous and subsequent treatment only in the actual perfusion. These samples were carefully neutralized with sodium carbonate, then made equally acid with acetic acid. To each specimen were added 1.5 gm. portions of a pulverized mixture of two

TABLE VI.

Melting Point of Osazones, Corrected.

Experiment No.	Locke's solution.		Pancreas perfusate.		Final heart perfusate.	
	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.
	°C.	°C.	°C.	°C.	°C.	°C.
16 (heart alone.)	204.4				204.9	205.4
17	205.59	204.09	201.58	204.09	200.57	203.58
18	204.05	205.02	203.55	206.52	202.62	206.02
19		205.02	201.51	205.52	200.50	205.52

parts of sodium acetate and one part of phenylhydrazine hydrochloride. As soon as these reagents were dissolved the specimens were filtered into test-tubes and placed in a boiling water bath for an hour. After cooling, the osazones were filtered off, washed with distilled water, and recrystallized from alcohol. The melting points were determined in capillary glass tubes by the usual method. Not the point at which the osazones sintered but the actual melting points were taken and the proper thermometer correction was made.

Table VI gives the results of twenty such determinations. The results show no essential diminution or increase in the melting points of the osazones yielded by the samples of original Locke's solution either before or after hydrolysis. Perfusion of a heart alone did not

materially lower the melting points of the osazones obtained. On the other hand, the osazones from the pancreatic and the heart-pancreas perfusates gave a distinct lowering of the melting points, while the same perfusates hydrolyzed gave osazones with melting points again approximately similar to those obtained from the Locke's solutions. Thus this osazone effect occurred only under those conditions in which the evidence from the optical rotation indicated a condensation of dextrose.

It is obvious that the osazones obtained in this way would consist of a mixture of glucosazone and the new osazones formed, and that the lowered melting points of the mixtures would be dependent upon the fact that the new osazones had a lowered melting point. The amounts of osazones available were so small relatively that no effort was made to isolate them in order to determine their independent melting points.

The only evidence from the osazones as to any qualitative or quantitative difference between the polymerization taking place in the pancreatic and heart-pancreas perfusions was in the slightly lower melting points of the latter. The general conclusion would seem to be that both the pancreas and the pancreas and heart bring about the formation of a substance or substances which yield osazones with lower melting points than glucosazone.

Bromine Analyses.

A possible method for studying the specificity of the heart and pancreas in their selection of dextrose was available in the property which bromine possesses of destroying dextrose but causing little if any change in levulose. Dextrose could thus be eliminated from a mixture with levulose and the amount of remaining levulose determined with the polariscope. The technique consisted simply in adding an excess of bromine to the acidified, hydrolyzed, protein-free specimens of fluid in glass-stoppered bottles, and, after thorough shaking, allowing them to stand for 7 days at 37°C. By using the hydrolyzed specimens the factor of any preliminary condensation was eliminated. All experiments were controlled by duplicate samples of the original Locke's solutions which had passed through an analo-

gous procedure. The bromine was removed by bubbling air through the fluids. Polariscopic readings then showed approximately the amounts of levulose which had been present in the original mixtures. The results of eleven experiments are collected in Table VII and the significant findings illustrated in Text-fig. 5. The first six experiments show that, by the method used, with dextrose alone there was always a small residue of positive rotation. In two experiments when a mixture of dextrose and levulose of approximately

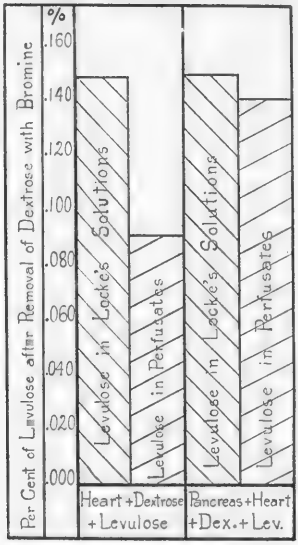
TABLE VII.

Experiments Showing the Results of Bromine Treatment of the Final Perfusates.

Experiment No.	Tissues perfused.	Sugar used.	After bromine treatment.			
			Locke's solution.		Perfusate.	
			Optical rotation with 4 dm. tube.	Sugar.	Optical rotation with 4 dm. tube.	Sugar.
				per cent		*per cent*
26	Heart.	Dextrose.	+0.10°	0.047	+0.10°	0.047
27	"	"	+0.04°	0.018	+0.09°	0.042
28	"	"	+0.10°	0.047	+0.09°	0.042
29	Pancreas.	"	+0.05°	0.023	+0.10°	0.047
30	"	"	+0.08°	0.038	+0.12°	0.057
3	"	"	+0.03°	0.014	+0.05°	0.023
16	Heart.	" + levulose.	−0.508°	0.139	−0.214°	0.059
31	"	" + "	−0.578°	0.159	−0.454°	0.124
17	Pancreas + heart.	" + "	−0.550°	0.151	−0.526°	0.144
18	" + "	" + "	−0.602°	0.165	−0.524°	0.143
19	" + "	" + "	−0.488°	0.134	−0.502°	0.137

constant composition was perfused through the heart, the amount of levulose, after bromine treatment, was considerably diminished in comparison with the original Locke's solution, similarly treated. This meant, in the two experiments conducted, that a certain proportion of levulose had been used by the heart. When, however, a pancreas perfusate with the same composition was fed to a heart, the amount of levulose was essentially undiminished, though the utilization of sugar had occurred to the usual extent. The utilization of sugar, therefore, when the heart and pancreas were combined,

must have been from the dextrose portion of the mixture. These results agree with the previous findings that the heart and pancreas have a specific action on dextrose.



TEXT-FIG. 5. The results in five experiments of treating the final perfusates with bromine for 7 days. A solution containing dextrose alone loses practically all of its sugar under this bromine treatment, while levulose is practically unaltered. When a heart is perfused with a solution containing practically equal parts of dextrose and levulose there is a considerable disappearance of levulose from the final perfusate as compared with the original Locke's solution. When the heart and pancreas, however, are combined in their effect, in spite of the increased sugar consumption there is practically no utilization of levulose.

DISCUSSION.

The following explanation seems to be most consistent with these facts and with those brought out in my previous work. The pancreas supplies to a perfusate some enzyme or enzymes which have a specific action on dextrose as compared with levulose, changing a certain portion of the dextrose to a simple form of polysaccharide. This polysaccharide has both a lower optical rotation and a lower

melting point of its osazone than dextrose. I should regard this sugar complex as either relatively unstable, being hydrolyzed during the reduction determination, or as having the same reducing properties as dextrose. When, however, the pancreatic perfusate is circulated through a living heart the optical rotation not only continues to diminish but a new change occurs. The reducing properties of the perfusate are now altered. Thus the effect of the heart seems to be the production of a change additional to that caused by the pancreas. The most probable explanation would seem to be a further polymerization of the sugar in the presence of the living heart to a more stable form with a diminished power of reduction. Neither of these apparent syntheses is brought about by an extract of the ground pancreas, by the perfusion of the spleen, the kidneys, or the heart alone, or by a combined perfusion of the pancreas and heart with levulose. As to the nature of these probable polysaccharides formed from dextrose, nothing definite can be stated, but the facts described tend to eliminate certain known sugars from consideration. Thus, because the optical rotations of the perfusates are diminished, maltose, isomaltose, saccharose, and glycogen cannot be considered, for each gives a greater positive rotation than dextrose. Lactose is also eliminated, for its rotation is identical with that of dextrose.

The supposition that synthetic changes are brought about by the pancreas or by the combined influence of the heart and pancreas, is in accord with numerous examples of the synthetic actions of enzymes which have been experimentally demonstrated by other observers. Thus, Hill (4) showed that maltase could synthesize a certain proportion of dextrose to what he thought was maltose. Lombroso (5) brought about a synthesis of fats by the action of pancreatic lipase. Taylor (6) obtained a protamine from its cleavage products by the action of trypsin. In my experiments some such synthetic action seems to be a more likely explanation of the results and one better in accord with our general knowledge of enzymes and sugars than the alternative suggestion of some essential change in the configuration of the dextrose molecule. The facts that osazones with lower melting points are obtained, and that the optical rotations of the perfusates and the melting points of the osazones can be reestablished to some extent by hydrolysis with weak acid, would be strong evidence that synthesis had occurred.

In this connection it is suggestive to note that the presence of more complex sugars than dextrose has been apparently demonstrated both in the blood and body tissues by various workers. Thus Pavy (7) and Lépine (8) by hydrolyzing blood increased its reducing properties. In a recent publication Palmer (9) has clearly shown that extracts of heart or skeletal muscle give a similar increase of reduction on hydrolysis. Again, a number of workers have obtained osazones with lower melting points than glucosazone from blood and muscle extracts. Panormoff (10) secured an osazone with a low melting point from extracts of dog muscle. From the blood Pavy and Siau (11) obtained an osazone which melted at 153°C., and suggested its identity with Fischer's "isomaltose." Osborne and Zobel (12) by more careful work concluded that this substance was maltose. Levene and Meyer (13), by combining muscle and pancreas extracts in the presence of relatively concentrated solutions of dextrose, isolated a biosazone which melted at about 200°C. The possible association of these facts with the present work is of interest.

The evidence previously advanced for the enzyme character of the substance obtained from the perfused pancreas is corroborated further in these experiments by the specificity of its action on dextrose as compared to levulose, and by the fact that the changes do not proceed to completion. Within recent years the specificity of enzyme action has received many proofs and in general it has been found that when an enzyme action is selective between two simple sugars, the selection is dependent upon the configuration of the sugar molecule rather than upon the presence of ketone or aldehyde radicals. It would seem possible that the selective action of the pancreas for the normal body sugar, dextrose, as compared to levulose also depends upon the molecular configuration rather than upon the fact that dextrose is an aldehyde and levulose a ketone sugar.

From the experiments here reported, it cannot be determined whether the specific sensitization of the heart for dextrose is dependent merely upon the preliminary change in the sugar produced by the pancreas. It seems probable that the action of the pancreatic enzyme does not cease with a simple polymerization but that it initiates a number of changes, the subsequent steps of which are dependent upon the interrelation of the enzyme with the living heart. As far as the heart alone is concerned there is a similarity in its action to that of an eviscerated diabetic animal as described by Macleod and Pearce (14). In both there is a certain apparent utilization

of dextrose, but for the isolated heart, at least, this utilization is non-specific, for it occurs almost equally well with levulose. In both, however, there is a certain fundamental incompleteness in the power to utilize sugar. With the heart, by supplying a pancreatic perfusate, a highly specific relation is established between it and the circulating dextrose which not only causes an immediate and rapid utilization of dextrose but also brings about a certain condensation of dextrose which can be initiated by the pancreas alone. This specific interrelation of the pancreas and heart to dextrose suggests that in normal sugar metabolism this pancreatic enzyme, or enzymes, may be necessary; that certain stages of synthesis and polymerization may be of importance as intermediate steps in carbohydrate utilization; and that when there is an insufficiency of the pancreatic function, though the body tissues are supplied with an abundance of dextrose which can be burned to a certain extent, yet the essential steps by which dextrose is prepared for normal utilization cannot take place.

SUMMARY.

When the pancreas of a dog is perfused aseptically with a Locke's solution containing dextrose in physiological concentrations, the optical rotation of the perfusate is diminished, but its reducing power is unaltered. This change also occurs if dextrose is added to a sugar-free pancreatic perfusate and the mixture incubated. These perfusates yield osazones with lower melting points than glucosazone, but when the perfusates are hydrolyzed with weak acid their optical rotations and the melting points of their osazones are increased. These changes do not occur with levulose, or with an extract of the pancreas and dextrose. When the heart, spleen, or kidneys are perfused with dextrose solutions hydrolysis of the perfusates does not increase their optical rotation or power of reduction.

When a pancreatic perfusate containing dextrose is circulated through a living heart not only do the above changes take place but, in addition, the reducing properties of the perfusate are altered. Hydrolysis of such a perfusate increases its reducing power, its optical rotation, and the melting point of its osazone. A heart does not cause this effect either alone or when perfused together with the

spleen or kidneys. Levulose perfused through the heart and pancreas is unchanged.

These phenomena are believed to be due to an enzyme or enzymes obtained from the perfused pancreas. The changes in optical rotation, in reduction, and in the osazones are accounted for by different degrees of dextrose condensation. While the living heart can destroy both dextrose and levulose to some extent, the experimental results suggest that the enzyme or enzymes derived from the perfused pancreas have a specific action on dextrose and are responsible for certain essential steps by which dextrose is prepared for normal utilization.

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CARRIAGE OF THE VIRUS OF POLIOMYELITIS, WITH SUBSEQUENT DEVELOPMENT OF THE INFECTION.

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Plates 55 and 56.

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The solution of the problem of the mode of infection in poliomyelitis has been attempted in various ways, with results which have led to the conclusion that the microbic cause is conveyed from one individual to another by personal contact. This belief is based upon clinical observation and experiment. Wickman first brought clinical proof, since supported by many independent observations, of the correctness of this generalization; and Flexner and Lewis, and later Kling and Pettersson, provided the experimental demonstration of its adequacy.

However, a considerable number of physicians and others still refuse to accept this explanation. They hold that the mode of infection remains undiscovered, or they account for it through some variety of insect transmission, also undetected. In recognition of the skepticism still prevailing, we have been led to describe in detail the experimental demonstrations of the carriage by healthy persons of the virus of poliomyelitis, to which may now be added our own successful inoculations. Our results include the demonstration, recorded for the first time, that a proved carrier of the virus may come down with acute poliomyelitis. This observation should serve to strengthen the position of those who accept as established the personal communication of the microbic cause, or virus, of the disease.

* Maintained by a special fund privately donated.

Previous Reports.

Wickman's[1] clinical studies may be said to have disseminated the view of the personal factor in the communication of the virus of poliomyelitis. He emphasized the occurrence and epidemiological importance of the non-paralytic or abortive cases, the first description of which is usually credited to him, and of healthy intermediaries, or bacillary carriers, who function as purveyors of the microbic agent. His study constituted a great step forward; but the first person to allude to non-paralytic cases of epidemic poliomyelitis is Caverly,[2] who records the occurrence of 6 cases among the total of 132 cases on which he based his report describing the Rutland epidemic of 1894.

Soon after Landsteiner and Popper's[3] experimental transmission of poliomyelitis, Flexner and Lewis[4] detected the virus in the nasopharyngeal mucous membrane of infected monkeys. This observation, soon confirmed by several independent bacteriologists, was followed by a study made by Kling, Pettersson, and Wernstedt[5] who injected into monkeys buccal washings from so called abortive cases and from healthy contacts. Their results were inconclusive, as the clinical condition produced was not typical of poliomyelitis, and the pathological changes described as present in the spinal cord were not characteristic of the disease. They explained the discrepancy by the supposition that the virus present in the abortive cases and healthy carriers was relatively avirulent. This view is repeated in their recent report[6] in which they describe an instance of healthy carriage of the highly active virus inducing paralysis and characteristic lesions. The first demonstration of the typical virus in the nasopharyngeal washings of healthy persons was, however, made by Flexner, Clark, and Fraser,[7] whose report follows in detail.

E. A., female, age 4 years and 4 months. The patient had been ill from Oct. 12 to 17, 1912. On the latter date she was admitted to the Hospital of The Rockefeller Institute for Medical Research, suffering from severe paralytic polio-

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[1] Wickman, I., Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907.

[2] In view of the importance which the non-paralytic cases have assumed in the epidemiology of poliomyelitis it is pertinent to quote Caverly, who states that paralysis occurred in 119 cases, 7 cases died before paralysis was detected, "and the remaining 6 had no paralysis, but all had a group of symptoms very common in the initial stage in those which were paralyzed, such as headache, fever, convulsions, or nausea, one or all" (*J. Am. Med. Assn.*, 1896, xxvi, 1).

[3] Landsteiner, K., and Popper, E., *Z. Immunitätsforsch., Orig.*, 1909, ii, 377.

[4] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 1140.

[5] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État à Stockholm*, 1912, iii, 5.

[6] Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.

[7] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201.

myelitis. She subsequently improved and was discharged. Oct. 28. The mother and father of the child were subjected to a nasopharyngeal irrigation with normal saline solution; about 150 cc. of washings were obtained. The fluid was shaken and passed through a Berkefeld filter; of the filtrate, 1.5 cc. were injected the same day into the sheath of each sciatic nerve and 140 cc. into the peritoneal cavity of a *Macacus cynomolgus* (Monkey A). Recovery from the anesthesia was prompt and the animal remained well until Nov. 11, when it was noted to be excited and to drag the right leg; the left leg was weak. Nov. 12. Right leg flaccid. A lumbar puncture yielded 2.5 cc. of fluid containing excess of white corpuscles. Nov. 13. The condition was unchanged; the animal was etherized. The organs generally were normal in appearance; the spinal cord was edematous. Microscopic examination of sections of the spinal cord, medulla, and interstitial ganglia revealed the characteristic lesions of poliomyelitis. The blood vessels and ground substance showed infiltrations with mononuclear cells; the motor nerve cells were degenerated and invaded by phagocytes.

Dec. 3. An emulsion of the glycerolated spinal cord and medulla was injected into each sciatic nerve and the peritoneal cavity of a *Macacus cynomolgus* (Monkey B) and a *Macacus rhesus* (Monkey C). Dec. 9. The *rhesus* monkey was noted to be excited. Dec. 10. Lumbar puncture yielded 3 cc. of turbid fluid containing excess of white cells. By Dec. 13, the legs were partially paralyzed; the animal was etherized. Microscopic sections of the spinal cord, medulla, and intervertebral ganglia showed typical infiltrative and degenerative lesions of poliomyelitis. The *cynomolgus* monkey became excited on Dec. 10, and on the 19th paralysis of the legs appeared. By Dec. 21 the arms and back were weak, and the paralysis was extending. Dec. 23. The animal was etherized. The general viscera appeared normal, but the spinal cord was both edematous and congested. The microscopic sections of the cord, medulla, and intervertebral ganglia showed typical infiltrative and degenerative lesions attended by neurophagocytosis. Subsequently the glycerolated specimens of the nervous organs of Monkeys B and C were used for inoculating still other monkeys, in which typical paralysis was induced.

The conclusion drawn by the authors from this demonstrative experiment was to the effect that the parents of E. A., neither of whom showed any symptoms of illness and who evidently were not suffering from poliomyelitis, harbored the virus of the disease in the nasopharynx. Hence the existence of the healthy carrier was thus established experimentally.

The next demonstrative experiment was supplied by Kling and Pettersson[6] who, in referring to their earlier failure to produce clinically and anatomically typical poliomyelitis with nasopharyngeal washings, attribute the failure to the injection of insufficient amounts of virus into the monkeys. They repeated the tests, using washings concentrated *in vacuo* with the Faust-Heim apparatus.

They started out by determining the heat lability of the active virus, and ascertained that a liter of fluid carrying an effective dose could be evaporated at

temperatures ranging from 35 to 38°C. to 200 cc. without losing its potency. They now obtained nasopharyngeal washings in amounts of 1 to 2 liters from healthy persons in contact with cases of acute poliomyelitis. In one instance in which the washings were taken from the healthy members of a family in which one member had recently died of acute poliomyelitis, the inoculation resulted successfully.

The patient was a male, age 41 years. The illness began on Sept. 10, the legs becoming paralyzed 2 days later. Death took place on the 4th day of illness from respiratory failure. The surviving members of the family consisted of the wife and three children ranging from 10 to 14 years, all remaining well. One day after the death of the father in a hospital, nasal washings were taken in distilled water from the surviving members of the family. The combined washings, amounting to 1 liter, were evaporated *in vacuo* to 75 cc., sodium chloride was added, and the mixture was filtered first through paper and then through a Berkefeld candle.

Sept. 20. 0.5 cc. of the filtrate was injected intracerebrally and 20 cc. were introduced into the peritoneal cavity of a *Macacus sinicus*. Oct. 2. The right leg and on the next day both legs and back were paralyzed, and death resulted. The microscopic sections of the spinal cord showed moderate perivascular and diffuse infiltration of the nervous tissue with mononuclear cells and neurophagocytosis. Oct. 3. A second *Macacus sinicus* was inoculated intracerebrally and intraperitoneally with an emulsion of the spinal cord of the first animal. On Oct. 13 the right leg and on the next day the left leg were paralyzed. Oct. 15. The animal was killed. Sections of the spinal cord showed typical infiltrative and degenerative lesions of poliomyelitis.

There can be no doubt, therefore, that in this family one or more healthy carriers of the active virus of poliomyelitis existed. That the result was not due entirely to the employment of concentrated washings is indicated by the failure to detect the virus in the washings obtained from the healthy associates of two other cases of acute poliomyelitis.

OBSERVATIONS.

In the two successful instances just reviewed, mixed washings were employed for inoculation. It is, therefore, impossible to state whether one or more of the healthy contacts of the cases of poliomyelitis were carriers. In the instance which we shall report the individuals were irrigated separately. The final result proved that more than one virus carrier was present, and it was demonstrated that such a healthy carrier may develop poliomyelitis. We may

therefore regard the chain of the mode of infection as now having been completed for the first time. The separate links may be defined as follows:

Case of acute poliomyelitis ⟶ contact carrier ⟶ second case.

A still further analysis would determine that through the contact carrier other carriers occur, among which a certain number of additional cases arise.

Poliomyelitis occurred in epidemic form in Washington County, Vermont, in the summer of 1917. From June 1 until September 1, 79 cases were recognized among the population of 45,000.

Carey P., male, age 16 years. The patient lived in the village of Waitsfield, 18 miles from Montpelier, where cases of poliomyelitis existed. No case of the disease had been discovered in Waitsfield. On June 2, 1917, he attended a ball game at Northfield where there were no cases, and returning home stopped in Montpelier for supper. Probably in the assembly at Northfield persons from the infected district were present. Until June 12 there were no symptoms of illness; on that day there was complaint of headache and pain in the back and legs. The patient vomited once. June 13. First seen by a physician who observed that the patient had fever, and treated him for a gastrointestinal upset. June 16. Extensive paralysis involving both legs, right triceps, intercostals, pectorals, and diaphragm. Lumbar puncture yielded clear fluid under pressure, containing 400 white cells per cmm. and excess of globulin. Death occurred on this date.

The family consisted of the father, age 59 years, mother, age 42, sister, Hazel, age 13, two brothers, Everett, age 10, and Dwight, age 7. The two younger brothers slept in the same bed, and in the same room with the elder brother Carey.

June 16. Everett and Hazel were given nasopharyngeal irrigation with distilled water, 60 cc. being obtained from the former and 100 cc. from the latter. 10 per cent of ether was added to each, and the fluids were sent at once to the laboratory. One of us had previously determined that ether inhibits bacterial development without injuring the poliomyelitic virus. The washings were treated separately as follows: Glass beads were added and they were shaken mechanically for 2½ hours. They were then centrifuged at high speed for 2½ minutes, and the supernatant fluid was passed through a Berkefeld N candle and concentrated *in vacuo* by the method already described by us[8] at 35°C. to 2 cc. The entire concentrate was injected intra-

[8] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

cerebrally into two *Macacus rhesus* monkeys (Monkey A (Everett) and Monkey B (Hazel)). The time elapsing between the collection and the injection of the washings was less than 6 hours.

We return briefly to the history of the two children. Everett had not been away from the village and was in usual health until June 13, the day after Carey fell ill. He also felt indisposed, showed a temperature of 102° F. and suffered from diarrhea, but did not vomit. However, he recovered quickly and subsequently on minute examination has shown no muscular weakness or abnormality of reflexes.

Hazel had not been away from Waitsfield. She had been entirely well at the time the washings were taken and remained well until June 21, at which time she complained of headache. She showed a temperature of 102° F. On June 22 her reflexes were exaggerated and stiffness of the back was present, but no muscular weakness was detected. Lumbar puncture was unsuccessful. The symptoms subsided gradually, but reexamination made on July 22 revealed partial paralysis of the left deltoid, right anterior tibial, and abdominal muscles. She had, therefore, suffered a mild attack of poliomyelitis.

Monkey A.—June 16, 1917. Inoculated. Remained well until June 29, when it was excitable, emitted staccato cries, and showed ruffled hair. The animal was noted to be clumsy in movement and unable to jump. June 30. Both legs were weak. July 4. The right leg was paralyzed and flaccid; the left leg and back were weak. The paralysis of the left leg and back, but not of the right leg, disappeared; the latter remained and contracture gradually set in. At the present time (Sept. 1) the contraction of the right leg is so marked that in moving about the animal does not touch the limb to the floor. On Aug. 8 blood was withdrawn for a neutralization test and at the same time an intracerebral inoculation was made with a large dose of virus proved active in another monkey; the result was negative. The animal, as is usually the case, having recovered from a recent infection, was resistant to reinoculation.

Monkey B.—June 17, 1917, 3 a.m. Injected intracerebrally with 1.5 cc. of the concentrated washings. Recovery from the anesthesia was immediate, and the first symptoms, consisting of excitability, ruffled hair, staccato cries, and partial paralysis of the right leg, were observed. June 26. The paralysis being stationary, the animal was etherized. The organs appeared normal to the naked eye. Microscopic sections revealed, however, marked typical lesions of poliomyelitis. They affected the spinal cord (Fig. 1), medulla (Fig. 2), and intervertebral ganglia (Figs. 3 and 4), and consisted of typical infiltration with mononuclear cells and nerve cell degeneration with phagocytosis.

Monkey C.—June 26, 1917. Injected intracerebrally under ether anesthesia with 2.5 cc. of a 20 per cent emulsion of spinal cord and medulla of Monkey B. July 7. The first symptoms were noted, consisting of ruffled hair and inclination of head to the left. July 8. The animal was ataxic and protected the right leg. July 9. Unable to jump; legs and back weak. July 10. Paralysis progressing. July 15. Etherized. The spinal cord showed typical focal lesions of poliomyelitis in which cicatrization was beginning.

These experiments leave no doubt that the washings, both from Everett and from Hazel, contained the virus of poliomyelitis. The instance of Hazel is of particular importance since in her case the virus was detected in washings taken 5 days before the first symptoms of what proved subsequently to be a mild attack of poliomyelitis set in. In other words, she was carrying the virus in her nasopharynx several days in advance of the appearance of any signs of illness. She constitutes, therefore, an example of a carrier of the virus developing poliomyelitis—the first one in which the demonstration has been proved experimentally.

The interpretation in the case of Everett is not so simple. When the virus was detected in his nasopharynx he had passed through a slight attack of illness, at about the same time with, and of about the same character as that of his brother Carey who died, but unattended by paralysis. The presumption is that Everett suffered from a non-paralytic or abortive attack of poliomyelitis. The detection of the virus in his case proves him not to have been a healthy, but a recovered carrier of the microbic cause of the disease.

The two children having been shown to be virus carriers, their nasopharyngeal secretions were tested by the method of Amoss and Taylor,[8] to determine whether they would neutralize an active poliomyelitic virus.

July 23, 1917. Washings with sterile water were taken from the children, and fractionally sterilized and mixed. To 15 cc. of the mixture were added 3.75 cc. of a Berkefeld filtrate of a 5 per cent stock glycerolated poliomyelitic spinal cord. After shaking, the combined fluids were permitted to remain at 37°C. for 24 hours. 1 cc. of the fluid was injected intracerebrally into a *Macacus rhesus*. No symptoms appeared until Aug. 4, when excitability, ataxia, paralysis of the right arm, and weakness of the back were noted. Aug. 8. Animal prostrate. Aug. 10. Died. The microscopic lesions were typical of poliomyelitis.

The mixed nasal washings failed, in this experiment, to neutralize the virus.

The youngest child, Dwight, age 7 years, was refractory and no washings were obtained from him on June 16 when they were taken from the other children. On June 18 he complained of being unwell. The symptoms were severe headache, stiffness of neck, exaggerated reflexes, but no diarrhea. Lumbar puncture yielded a fluid containing 500 white cells per cmm. and an excess of globulin. Immune poliomyelitic serum from recovered cases of the disease was administered intraspinally, intravenously, and subcutaneously: 24 cc. were given intraspinally, 30 cc. intravenously, and 39 cc. subcutaneously. Recovery was prompt, with a slight paralysis of the right anterior tibial muscle. Nasopharyngeal washings were, however, obtained on September 4, which after filtration and concentration were inoculated into a *Macacus rhesus* (Monkey D). The monkey remained well.

DISCUSSION.

This series of cases of poliomyelitis in one family, with the circumstances surrounding their origin, forms an instructive illustration of the mode of infection of the disease as brought out by the clinical and experimental study.

In the first place, one child only—the eldest boy, Carey—was exposed in a locality in which poliomyelitis was epidemic. The exposure took place on June 2. Immediately afterwards he returned home, to a village in which no previous case of the disease had occurred, and mingled freely with his younger brothers and sister. The contacts may be considered to have been intimate in that the three male children slept in the same room, two of them in the same bed.

The incubation period in Carey's case was 9 or 10 days, as he was taken ill on June 12. His brother Everett, 6 years younger, developed symptoms 1 day later and passed through what was probably a non-paralytic attack of poliomyelitis. He may be considered as having been infected by Carey some time during the incubation period, and to have exhibited a shorter incubation than his brother. The youngest brother, Dwight, was also freely exposed to both older brothers and exhibited symptoms passing into those indicative of

poliomyelitis 5 or 6 days later than his brothers. Finally, Hazel, the sister, in age between the two older brothers and possibly less freely exposed, developed symptoms and muscular weakness last of all and about 10 days after the eldest brother. The incubation periods of the cases, therefore, probably were 10 days or less, and the order of the attacks was such as to indicate successive infection and not a common one.

The second feature worthy of emphasis is the detection in this one family of two carriers of the poliomyelitic virus by the inoculation test. One (Everett) was discovered to be a carrier probably following a non-paralytic attack. In the instance of Hazel there is no doubt, first that she was discovered to be a healthy carrier, and second that she developed typical poliomyelitis during the period of carriage. Incidentally the nasopharyngeal secretions of Hazel and Everett failed to neutralize the poliomyelitic virus.

If the view that the mode of infection in epidemic poliomyelitis is by way of the nasopharyngeal mucous membrane and is brought about or greatly facilitated through the operation of healthy carriers of the virus, we may well consider whether in the final analysis every case of the disease does not develop from a carrier. At first this may seem startling, and yet it merely means that after contamination of the nasopharynx with the virus, an intervening period exists during which persistence, multiplication, and invasion of the virus take place. In not all contaminated persons does this process become complete; in some the virus may merely persist for a time, in others it may multiply in the nasopharynx (these constitute the healthy carriers of greater or less endurance), while in the exceptional few invasion also occurs. In the latter, symptoms arise, and such individuals compose the class of poliomyelitis cases.

SUMMARY.

A family group containing four children of whom all showed in varying degree symptoms of poliomyelitis is described. The source of infection and periods of incubation have been followed. Two of the children were proven by inoculation tests to carry the virus of poliomyelitis in the nasopharynx. Of these, one was detected to be

a carrier after recovering from a non-paralytic attack of the disease, and the other was discovered to be a carrier about 5 days before the initial symptoms, attended later by paralysis, appeared. The original case from which the three others took origin was fatal; the youngest child, after quite a severe onset, was treated with immune serum, and made a prompt and almost perfect recovery. The nasopharyngeal secretions of two of the cases, taken 1 month after the attack, proved incapable of neutralizing an active poliomyelitic virus.

The proposition is presented that every case of poliomyelitis develops from a carrier of the microbic cause, or virus, of poliomyelitis.

EXPLANATION OF PLATES.

PLATE 55.

FIG. 1. Spinal cord of Monkey B, showing perivascular infiltration and neurophagocytosis. × 90.

FIG. 2. Medulla of Monkey B, showing diffuse mononuclear infiltration, nerve cell degeneration, and neurophagocytosis. × 230.

PLATE 56.

FIG. 3. Intervertebral ganglion of Monkey B, showing infiltrative changes and nerve cell invasion. × 120.

FIG. 4. Intervertebral ganglion of Monkey B, showing mononuclear infiltration, nerve cell degeneration, and neurophagocytosis. × 240.

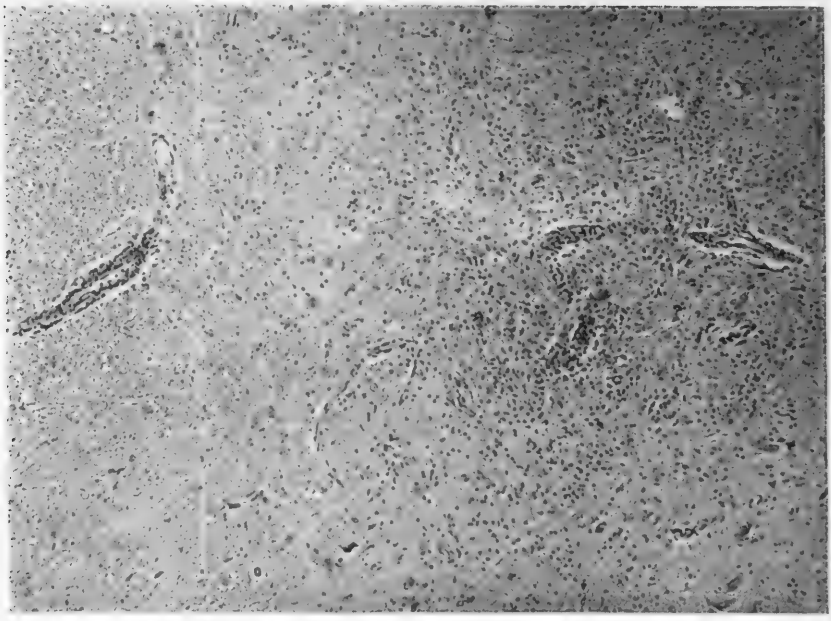

FIG. 1.

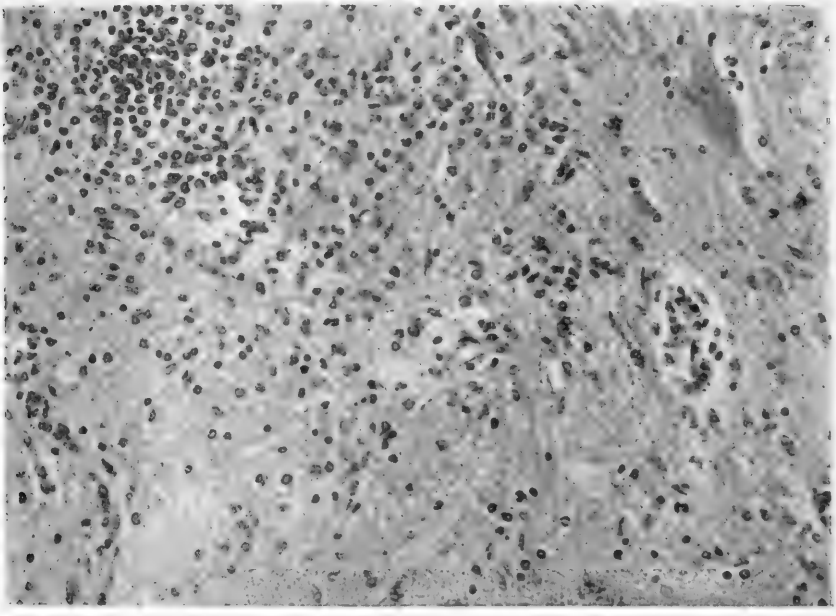

FIG. 2.

(Taylor and Amoss: Carriage of the virus of poliomyelitis.)

FIG. 3.

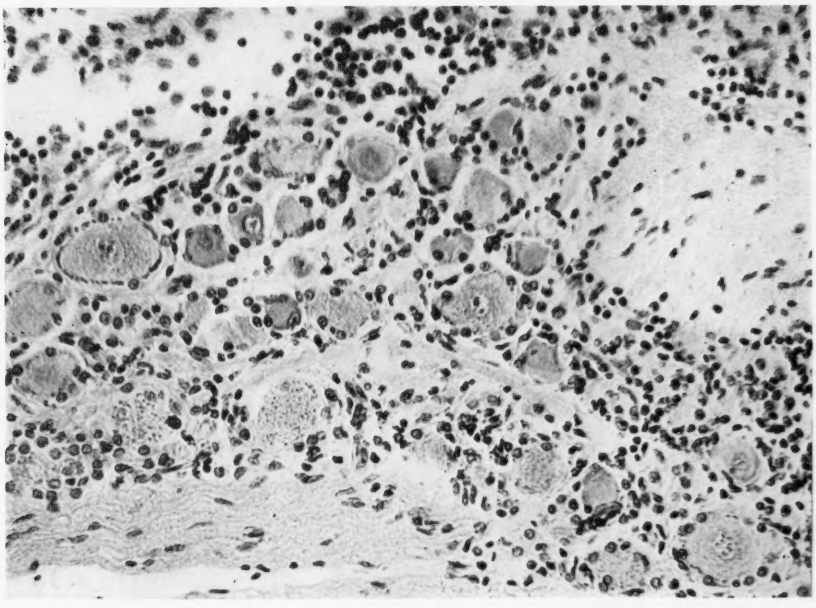

FIG. 4.

(Taylor and Amoss: Carriage of the virus of poliomyelitis.)